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AN ANNIVERSARY TRIBUTE TO THE MEMORY OF THE LATE WILLIAM HENRY HOWELL

ALAN M. CHESNEY

FOREWORD

On Friday, February 24th, 1961, there was held in the east lecture room of the Basic Science Building of the School of Medicine of The Johns Hopkins University, at 725 North Wolfe Street, Baltimore 5, Maryland, a meeting expressly planned to honor the memory of the late Dr. William Henry Howell, A.B., 1881, Ph.D., 1884, The Johns Hopkins University.

The meeting was sponsored jointly by the School of Medicine and the School of Hygiene and Public Health because of Dr. Howell's long and important connection with both of these institutions. It was held in conjunction with the 1961 Biennial Meeting of the Johns Hopkins Medical and Surgical Association, since the date of that meeting coincided more closely than that of any other meeting with the 100th anniversary of Dr. Howell's birth which took place in Baltimore on February 20, 1860.

A number of pupils and friends of Dr. Howell, together with members of his family, were present on the occasion and the following program was carried out.

Presiding:
PHILIP BARD
Speakers:
ALAN M. CHESNEY
"Early Years—Faculty of Philosophy"
L. EMMETT HOLT, JR.
"Middle Years—Faculty of Medicine"
ANNA M. BAETJER
"Late Years—Faculty of Hygiene and Public Health"

An exhibit of photographs, letters, books and memorabilia relating to Dr. Howell, arranged by Mrs. Janet Koudelka of the staff of the Welch Medical Library, was shown at the same time.

The text of the three papers follows.

AN ANNIVERSARY TRIBUTE TO THE MEMORY OF THE LATE WILLIAM HENRY HOWELL: EARLY YEARS

ALAN M. CHESNEY

WILLIAM HENRY HOWELL was a Marylander in every sense of the word. He was born in Baltimore on February 20, 1860 and, except for a period of four years, lived all of his life in that city. Both his father and his mother were born in the country side of Maryland and their ancestors settled in Maryland in early colonial times. On his mother's side he was descended from a Scot by the name of MacGregor who fought against Cromwell's army, was taken prisoner at the battle of Worcester and banished to Maryland as an indentured servant in 1651. As part of his punishment for supporting the Royalist cause this MacGregor was forced to change his name, which he did, taking the name of Magruder and locating in Southern Maryland. He seems to have prospered and managed to free himself from the terms of his indenture within a year. He was married twice and Dr. Howell's mother was descended from the first wife, Margaret Braithwaite, a cousin of the 2nd Lord Baltimore. From the standpoint of lineage, therefore, Dr. Howell was fully entitled to be considered a Marylander.

From the standpoint of education, also, Dr. Howell can be considered a product of his native state, for all of his formal education was obtained in the public schools of Baltimore and in The Johns Hopkins University. After completing grammar school he entered the Baltimore City College, as the local boys' high school was called in those days, but he did not graduate from that institution because, during his third year of enrollment, he was offered and accepted a paid post as assistant to the "professor" of natural science in the "college". This post required Howell to assist in the preparation for the classes in chemistry, but it also, according to his own testimony, gave him the opportunity to perform, on his own initiative, many experiments illustrative of fundamental chemical principles, such as, for example, Wöhler's classical experiment of converting ammonium cyanate into urea. Thus he had the opportunity to gain, at first hand, an acquaintance with the methods by which scientific knowledge is acquired.

The young Howell, however, did not at once decide to embark upon a career devoted to scientific investigation. That decision came later. His first inclination was to become a physician, and on February 5, 1879, shortly before his nineteenth birthday, he wrote a letter to Dr. John Shaw Billings, at the Office of the Surgeon General of the United States

Army in Washington, telling of his desire to study medicine and asking several specific questions in relation to the Johns Hopkins Medical School.

His reason for selecting Billings as the person to whom to address his questions, instead of some administrative officer of the University, is not hard to explain. Billings had been selected by the Trustees of The Johns Hopkins Hospital in July 1876 to develop a plan for the construction of that institution, and in the autumn of 1877 he delivered before the University a series of twenty lectures on the history of medicine, on medical legislation and on medical education. In the last lecture of the series, which was devoted to medical education, he asked the following question: "In view of the present condition of Medical Education in this country, how should the Johns Hopkins University organize its Medical Department?" That particular lecture was reported in the local press and published later as a separate document, so that it is not surprising, perhaps, that Howell should have first approached Billings with his questions.

In his letter Howell made it clear that he wanted to be able to study medicine in The Johns Hopkins University rather than in one of the medical schools existing in Baltimore at the time, and he asked the following three questions:

"First.—Would it be possible for me to skip the first two years of the preliminary course, and stand an examination at the beginning of the session in September 1880, directly for the 3rd year?

Second.—Could I obtain a free scholarship for four years?

Third.—Will the Medical School proper be ready to open at the beginning of the session in September, 1881?"

Back of these three questions was the fact that Howell could not see his way clear to finance both a college and a medical school education. Billings replied promptly, referring Howell to President Gilman for the specific answers to his questions but took occasion to emphasize the importance of his taking the preliminary medical course before entering upon the study of medicine, and then went on to say that there was nowhere any better place to take it than at the "Hopkins University". Howell at once wrote to Gilman, had a satisfactory interview with him which Howell himself has described, and was finally admitted to the University in the autumn of 1879 as a fully matriculated candidate for the degree of bachelor of arts, enrolled in the three-year preliminary medical course. He was given credit for one year of work toward the completion of that course, and as a result he could look forward to receiving his bachelor's degree in two years.

It is of interest that in seeking to enter upon a medical career Howell was not following any family tradition or example. Neither his father

nor his brothers nor his uncles had ever gone to college and he was therefore striking out on his own. The fact that ultimately he abandoned his intention to become a physician and chose instead a university career in physiology was undoubtedly due to the influence of the new environment to which he was exposed in The Johns Hopkins University, plus the fact that there occurred an unexpected delay in the opening of the Hopkins Medical School.

During his first year as an undergraduate student Howell took courses in general biology, osteology, and general and analytical chemistry. Since the course in general biology was given by the head of the department himself, Henry Newell Martin, Howell came in contact at once with that brilliant individual who undoubtedly exerted a great influence upon his subsequent career.

Martin had come to the University at its opening, only three years before, as its first professor of biology, and was himself a young man, being only twenty-eight years of age at the time of his appointment to the chair. A native of Ireland and the son of a Congregationalist minister, he had studied at University College, London, and also at Cambridge, where he was a pupil of the celebrated physiologist, Michael Foster. He was by all accounts a brilliant teacher and investigator, and he quickly established an outstanding department at the Hopkins.

This department, although labeled "biology", was oriented more toward the study of mammalian physiology than toward what is now included under the term "general biology", and this orientation was entirely intentional on Martin's part, as we shall see later on.

Of Howell's life as an undergraduate student at the Hopkins in those early days little has been recorded, but Dr. Henry M. Thomas, Sr., a fellow-student enrolled in the preliminary medical course along with Howell, has left us the following bit concerning his athletic prowess:

"In 1879 William H. Howell came from the Baltimore City College and entered the chemical-biological course and began a career which was to mean much to the university and medical school. . . . I, in my capacity as experimental animal was entered in the university the same year, and I can well remember Howell as the model student and also on the football field where he made up for his light weight by the accuracy and neatness of his tackling."[1]

Howell, as expected, obtained his A.B. degree after two years of study, during which he completed major courses in biology and chemistry and

[1] Bulletin of the Johns Hopkins Hospital, 1919, vol. 30, page 187.

minors in physics, German and philosophy.[2] The commencement exercises were held on June 6th (1881) and sixty years later Howell said of that occasion: "Never have I experienced such an all-pervading, quiet but deep sensation of satisfaction as on that day when I received my bachelor's degree in Arts. It seemed to me that I was being admitted into that goodly company of scholars I so much admired—and in fact that proved to be the case, for since that time my associations have been mainly with scholars, meaning by that term men and women who have a love of learning and the desire and ability to promote it."[3]

Upon graduation as a bachelor of arts Howell was awarded a graduate scholarship for one year which he accepted. Up to this time he had been able to pay his way in the University with the money which he had himself earned, but we know from his letters to Billings and to Gilman that he would be in need of money if his education was to be a lengthy one. The receipt of this scholarship and a fellowship awarded later on by the University, enabled him to continue in graduate study. Years later Howell was to say: "In this way my original intention to become a practitioner of medicine was diverted and I entered upon the career of a teacher and investigator."[4] Surely no scholarship or fellowship bestowed by The Johns Hopkins University has ever returned more to the institution itself than those which were bestowed upon William Henry Howell.

The Hopkins department of biology, when Howell enrolled in it as a graduate student, consisted, in addition to the head, of the following four persons of faculty rank:

William K. Brooks, Associate.
William T. Sedgwick, Associate.
Henry Sewall, Associate.
Edmund B. Wilson, Assistant.

All four of these men achieved eminence in their chosen fields as time went on, and, together with Martin they must have constituted an inspiring group with which to be associated.

During the three-year period of his study for the Ph.D. degree four papers appeared from the biological laboratory of the Hopkins with Howell's name and that of another student as co-author, and a fifth with his name alone. All but one of these papers dealt with some phase of

[2] We know, also, that he dissected a human cadaver at the University of Maryland under the supervision of Dr. Randolph Winslow, later professor of surgery in that institution.

[3] Bulletin of the Johns Hopkins Hospital, 1941, vol. 68, page 302.

[4] Bulletin of the Johns Hopkins Hospital, 1941, vol. 68, page 305.

the circulation of the blood. The thesis which he submitted for the Ph.D. degree dealt with the coagulation of the blood, the subject with which his name will forever be associated. The title was "Experiments upon the Blood and Lymph of the Terrapin, and the origin of the Fibrin formed in Coagulation". The degree was conferred upon him on June 5, 1884, and it was stated that his "principal subject has been Animal Physiology and Histology, the subordinate, Chemistry."[5]

It was during Howell's last year as a graduate student that the "new" biological laboratory of the Hopkins was opened. Prior to that event the department of biology had been housed in buildings which it shared with other departments and it must have been considerably hampered by lack of space. With the acquisition of a new three-story building all its own, however, which was placed at the southeast corner of Eutaw and Little Ross Streets, the department was no longer so handicapped. Howell, therefore, when he accepted the post of assistant in biology which had been tendered him by the university authorities upon his graduation as a Doctor of Philosophy, had the satisfaction of having entirely new and well equipped quarters in which to begin his instructional duties as a fledgling member of the Hopkins faculty.

The new building had been formally opened on the evening of January 2, 1884, with an address by Martin in which he expressly stated that the building had been constructed "primarily to afford facilities for instruction and research in physiology; and secondarily, similar opportunities in allied sciences, as comparative antomy and botany, some training in which is essential (and the more the better) to everyone who would attain real knowledge of physiology."[6]

Martin recognized that the resources of his department at the time did not permit the development of both physiology and morphology on an adequate basis and, since it seemed to him better to do one thing well rather than to do two things imperfectly, he chose to place major emphasis upon the development of physiology and planned the building to that end.

His choice of physiology as the major subject for development in the department coincided with his own interests, certainly, but it was also influenced undoubtedly by the close relationship of that subject to medicine, and by the fact that the Hopkins authorities fully expected that the University would in time have a medical school of its own.

Martin was fully aware, as his address shows, of the great contributions which physiology was in a position to make to other medical disciplines,

[5] Johns Hopkins University Circular, 1884, No. 31, page 119.
[6] Johns Hopkins University Circular, 1884, No. 30, page 87.

notably pathology and therapeutics; moreover, he was greatly interested in seeing that students entering medical schools were properly prepared for the task that lay ahead of them, and he knew that his department could make a significant contribution toward that endeavor. Indeed, he stated categorically in his address; "I regard it as the most important of my duties, to prepare students to enter medical schools in this city, or elsewhere". The study and teaching of physiology was, then, the keystone of the Hopkins department of biology in its earliest days, and it was in that environment that Howell, now twenty-four years of age, began his career as a teacher.

It is of interest, perhaps, that that same year, 1884, witnessed the appointment of William H. Welch as the first professor of pathology in The Johns Hopkins University. No one at that time, certainly, could have foreseen that these two men, Welch and Howell, would years later be so closely associated in the launching of two new schools in that same university.

Howell's initial task in the department was to assist in the laboratory work which formed a major part of the course in general biology for those who were beginning the study of that subject. His performance must have been entirely satisfactory for at the end of the first year he was promoted to the grade of associate and we find him, during his second year in the department, conducting a special course of his own, labeled "Physiology of Respiration", to eight students. From that time on the story is one of increasing responsibility for the instruction regularly given in the department. Thus by his third year he was conducting special courses in mammalian anatomy, vertebrate histology and animal physiology, in addition to assisting in the general laboratory course, and this heavy burden of instructional work he continued to carry for the remainder of his stay as a member of Martin's department.

In spite of the heavy teaching responsibilities which devolved upon him, Howell found time to carry on his own investigations and three papers were published by him during the five years in which he was in the Hopkins department of biology.

Howell greatly admired Martin and certainly the relations between the two men were most cordial. Nobody was more distressed than Howell by the unhappy events which clouded the older man's later years.

In 1887 Howell married Miss Anne Janet Tucker of Baltimore, and in the following year he was appointed associate professor of biology. One year later he was called to the University of Michigan to take over the chair of physiology which his friend and former associate at Hopkins, Henry Sewall, was giving up because of failing health. He accepted the

call and so, at the age of 29, his first Hopkins period came to an end. We have it on the authority of his daughter, Dr. Janet Howell Clark, that her father always looked back upon this first Hopkins period as the golden period of his life. However that may be, we of Johns Hopkins may rejoice that it proved not to be his only Hopkins period, but that after four years of sojourn in two states of the North, it was given to him to return in 1893 to his native state, and to the University which he had already served so well.

ACKNOWLEDGMENT

The author wishes to acknowledge his indebtedness to Dr. Janet Howell Clark for her invaluable help in the preparation of this paper.

AN ANNIVERSARY TRIBUTE TO THE MEMORY OF THE LATE WILLIAM H. HOWELL: THE MEDICAL SCHOOL YEARS (1893–1912)

L. EMMETT HOLT, JR.

The period which I shall talk about was largely before my own time. It was only toward the end of it that I knew Dr. Howell and had the privilege of working in his laboratory. For the early part I have had to turn to the record. That very extraordinary group of men that formed the first Hopkins faculty—Mall, Abel, Howell, Walter Jones, Osler, Halsted, Kelly and Whitridge Williams had been selected largely by Dr. Welch who remained the presiding genius and served as dean for the first few years. They were all stars of the first magnitude—a medical faculty of investigators as well as scholars and they made Johns Hopkins a unique center of learning for several decades. How much of a part Dr. Howell played in the affairs and the development of the school is difficult to say. His calm wisdom and balanced judgment must have been quickly appreciated by his colleagues and in 1899 he succeeded Dr. Welch as dean of the school, a post he held until 1911.

The department of physiology prospered. The staff was small but its efforts were supplemented by a succession of students, no doubt attracted by the extraordinarily clear and stimulating lectures in physiology. The publications of the department covered a wide range of subjects: methods of measuring the blood pressure, shock, the physiology of sleep, the cause of heart beat—its chemical and nervous regulation, the function of the pituitary gland. The textbook of physiology was written during these early years. From 1910 on the problem of blood coagulation was Howell's absorbing research interest, one which continued for the rest of his life. Many of us who associate his name so closely with this last problem are perhaps not aware of the major contributions he made in other areas. I had to look up the record to discover that he was the first investigator to separate the function of the anterior and posterior lobes of the pituitary, demonstrating clearly that only posterior lobe extracts affected the circulation. And I wonder how many cardiac surgeons of today who employ potassium to induce cardiac arrest realize that they owe to Howell the fundamental knowledge of the effect of inorganic ions upon the heart.

I learned about Dr. Howell when I came to the medical school in the fall of 1916. In the minds of upper classmen, our chief mentors, the physiology course was glamorous above all others. It was the best taught

course in the medical school and also the most important. How you did in physiology might make or break your subsequent career. And Dr. Howell, of course, was the world authority on blood clotting. One very bright student whom I knew had actually penetrated the department and was working on a mysterious substance known as metathrombin. His name was Arnold Rich. Small wonder that my eyes were turned in that direction. I had some spare time on my hands, having been credited for a laboratory course in biochemistry at Harvard and I finally summoned up my courage and went over to see Dr. Howell to see if he could give me something to do in my spare time. He smiled at me, "Well, Mr. Holt, you know we're mostly interested in blood clotting around here; would you like to work on that?" Would I. My first assignment was to study some leaves from the Brazilian jungle which Dr. Watson Sellards had sent to Dr. Howell. They were thought to contain a mysterious coagulant, and they were used by the natives to apply to fresh wounds. My job was to extract and try to identify the active principle. I learned the various techniques for testing coagulation factors and then set to work on the leaves; they were dry and crumbly by that time; they were ground up, extracted and tested, but the result turned out to be just the opposite to that expected; they contained not a coagulant but an anticoagulant—an antithrombin. We then went on to study other leaves—including tea leaves and sure enough, they had similar anticoagulant properties; eventually it was discovered that tannic acid was the active component. The work was never published, perhaps because something more interesting developed which was my next job. In an attempt to isolate a coagulant phosphatide from liver another student, Jay McLean, had likewise observed anticoagulant rather than coagulant properties. He had left the laboratory and I was assigned the task of purifying the anticoagulant liver fraction. I spent hours reading German monographs on phosphatides, extracting dog livers, precipitating and re-extracting and purifying the material. Dr. Howell named it heparin; its place in the cogulation picture was duly established, even though its chemical nature was not well defined. Being a physiological substance it was much more interesting than a Brazilian leaf. In subsequent years Dr. Howell obtained purer, phosphorus-free preparations of heparin and identified carbohydrate groups in it; its final constitution was established by Jorpes in Sweden.

The final examination in physiology came in the middle of our second year; it was a major hurdle for all the class but for those of us who worked in the laboratory doubly so. We couldn't let Dr. Howell down. And Franklin Hanger and I who worked together came very close to memoriz-

ing Howell's physiology. As soon as the examination was finished I turned up in the lab for work, but Dr. Howell sent me home saying, "I think you've had enough physiology for today."

A few days ago I looked up the article on blood coagulation in what had once been Howell's Physiology and was still listed as the "eighteenth edition of Howell's Physiology" in the subtitle. It was a good article but it was something of a shock to find no mention of Howell in it and no reference to any of his painstaking work which extended over a period of thirty-five years. Had the work vanished like that of Ozymandius of Egypt? Had it all been done in vain and was everything disproved by subsequent work? I don't think so. New coagulation factors have been discovered and theories of coagulation have had to take them into consideration, but many of his concepts remain and much of his work provided solid building stones for those who came later. He made real progress in purifying and identifying a number of clotting factors, developed the first practical method of measuring prothrombin and antithrombin and identified cephalin as the important thromboplastic constituent of brain tissue. He developed the concept that circulating anticoagulants were responsible for keeping the blood fluid in the blood vessels and demonstrated the presence of two of them—antithrombin and heparin—in normal blood. From the practical point of view I am inclined to think that the discovery of heparin, for which he was really responsible, was perhaps his most important contribution.

Dr. Howell was a born investigator; he worked long and hard at his problems; he was highly intelligent, critical and imaginative but his motivation seemed to differ from that of most investigators. His drives did not seem to be emotional; if they were the emotion was always perfectly controlled. There was nothing temperamental in his make-up. He was critical of the shortcomings of others but always kindly in his evaluation of them. Personal ambition was certainly not a driving force; it was rather an intense and pure curiosity. Such a man could have no scientific enemies. He was loved and revered by his fellow physiologists and was repeatedly elected as president of the American Physiological Society of which he had been a founder.

Perhaps posterity will overlook Dr. Howell's scientific contributions. With the newer tools of today they are being buried by new knowledge, a fate that is reserved for all but a few epoch making discoveries. What will remain as his most important contribution is his work as a teacher. More than a generation of American doctors was raised upon his textbook, written with a clarity and simplicity that carried the message to every reader. And no student of the twenty-five classes he taught at Johns

Hopkins can forget his clear and stimulating lectures; never dramatic, they held the audience because the enthusiasm and interest of the lecturer was contagious. The small group who were privileged to have close contacts with him will carry all their lives the inspiration of his qualities of mind and character. I wish I had the eloquence to bring him to life. Instead I shall close by repeating some words of Dr. Park, spoken at a dinner twenty years ago in Dr. Howell's honor. "As the years go by," he said, "I long to be more like him, to possess his elevated standards, his keen social consciousness, to have his lucidity of mind, the power to approach each question with such clearness, personal detachment and fairness, and, finally, to possess his calm philosophy and his ability to live in the light of it."

I am sure that those of us who knew Dr. Howell will always carry with us a measure of inspiration from him. He was a man apart. If our meeting today can convey something of him to those who did not know him, I think it will have achieved its purpose.

AN ANNIVERSARY TRIBUTE TO THE MEMORY OF THE LATE WILLIAM H. HOWELL: LATE YEARS—FACULTY OF HYGIENE AND PUBLIC HEALTH

ANNA M. BAETJER

Dr. William Howell's connection with the School of Hygiene and Public Health began in 1916 when he was appointed to assist Dr. William Welch in the organization of this new school which, in accordance with the grant from the Rockefeller Foundation, was not connected with the medical school but was a separate institution. Dr. Howell was appointed assistant director and also served as professor of physiology and head of the department of physiological hygiene when the school opened in the fall of 1918. In November 1926, when Dr. Welch resigned from the school to inaugurate the chair of the history of medicine, Dr. Howell took over the directorship in addition to his other duties. He retained this position until his retirement in June 1931 at the age of 71 years when he was named director emeritus and professor emeritus of physiology.

In considering Dr. Howell's career in the School of Hygiene and Public Health, two questions come to mind at once: why did Dr. Welch choose Dr. Howell, a physiologist, to assist him in organizing a school of public health; and, even more interesting, why did Dr. Howell accept this new assignment?

The answer to the first question is found in a short history of the Department of Physiological Hygiene written by Dr. Howell in 1939, six years before his death. The opening paragraph of this history is quoted here. "In organizing the School of Hygiene and Public Health it was Dr. Welch's intention to restore physiology to the place it had formerly occupied at the time of Pettenkofer, in the prebacteriological days, as one of the fundamental subjects upon which scientific instruction in hygiene must be based. This intention explains probably the selection of Dr. Howell, a physiologist, to assist in the work of organization of the new school, and is implied also in the double title given to the School, namely a school of hygiene and public health . . . The actual organization of the physiological side of the curriculum of the new school was left to Dr. Howell." With his customary modesty Dr. Howell failed to add that Dr. Welch undoubtedly chose him over all the other physiologists of that day because of his well recognized success as an administrator, as an inspiring teacher and as a careful and critical scientist.

The answer to the second question is perhaps more difficult. Upon first consideration it seems surprising that Dr. Howell would accept this new

assignment when he already held the professorship of physiology in a leading medical school in the country and was in the midst of his most important research. Why, then, would he relinquish this post for a school of hygiene and public health in an era when the entire interest in public health was centered on microbiology and the control of communicable diseases? Fortunately, an answer to this second question was clearly set forth by Dr. Howell in a paper which he published in 1921[1] under the title "Scientific Research and Public Health." After discussing the fact that modern hygiene was based chiefly on the control of infectious diseases, he stated that hygiene was equally concerned with all those physical and chemical forces in the environment that react upon the body from within and from without. He continued with the statement, "The maintenance of health requires an unending series of adaptations to changing conditions. . . . The mere fact that the machinery runs so well through so many years is a demonstration of the completeness of the physiological adaptations." Dr. Howell then pointed out that as life continues, failures in adjustment and adaptation appear which develop into serious pathological defects. "Senescence may be regarded as a gradual developing loss in the power of adaptation of the tissues to their internal environment . . . but by unhygienic living, by deliberate or ignorant disregard of the rules of health, we can bring about a premature disruption of the delicate adjustments of the body. A perfect system of preventive medicine would detect these deviations from the normal at the very beginning and would correct them by the application of hygienic measures . . . We are very ignorant in regard to what might be called the rules of right living, that is to say the kind of living that would maintain the functions of our body at the highest standard of efficiency. The working out and formulating of these rules constitutes the main objective of so-called personal hygiene." Dr. Howell then noted that unfortunately the subject of personal hygiene had been discredited by the publication of foolish and fanciful methods of preserving health and that "more harm has been done through the misdirected efforts of propagandists whose zeal is greater than their knowledge, and who in their effort to get something across to the public which in their eyes seems good are guilty of much virtuous lying. Personal hygiene to most persons means something dreadfully bromidic like brushing the teeth or daily baths . . . In reality personal hygiene is a subject of the greatest importance which has been much neglected . . . The expectation of life at birth has been increased during the last fifty years, largely through control of the infectious diseases of early life. But most of us would place an equal or higher value

[1] Transactions of the Royal Canadian Institute, Toronto, 13: #2, 1921.

upon the kind of knowledge that would enable us to avoid those disabling defects and illnesses that bring about chronic invalidism or impair our physical efficiency, without killing us outright . . . Science has knowledge and methods which if applied intelligently should add something of real importance . . . in regard to the rules of living . . . In all schools of Hygiene and Public Health there should be physiological departments organized with this point in view . . . In our new school at Baltimore recently established I am glad to say that we have borne in mind that work in public health has in the last analysis two great objectives, the control of communicable diseases on the one hand and the preservation of normal health on the other, and provision has been made for instruction and research along both lines."

From this account it would seem that Dr. Howell was attracted to the School of Hygiene because he saw there an opportunity and a challenge to direct research in physiology toward promoting good health rather than toward the understanding and treatment of disease. To him, keeping people well was a noble objective in keeping with his philosophy of life. Thus 40 years ago he saw the need for physiological research as a basis for the prevention of chronic diseases, for understanding the effects of the environment on health and for determining the fundamentals of sound living—trends in public health which today are only beginning to attract the interest of the medical profession.

Another reason, according to his daughter, for Dr. Howell's acceptance of the position at the School of Hygiene was that he enjoyed administrative work and welcomed the opportunity to help organize and administer this new institution. There is no doubt that Dr. Howell played the major role in the initial organization and administration of the school as a whole. The main objects of the school, as stated in the Preliminary Announcement[2], were: 1) "to establish courses for the training of qualified persons in public health work" and 2) "to promote investigative work in hygiene and preventive medicine and provide opportunities for training investigators in these subjects." Although Dr. Howell recognized the need for the practical training of public health personnel, he thought that the object of such teaching should be to impart a knowledge of principles rather than methods and facts. His chief contribution to the public health field, however, was not through the teaching program he initiated but through his emphasis on the second objective of the school's program, i.e., investigative work. During the last years of Dr. Howell's administration, because of the demand for trained public health doctors, considerable pressure was exerted upon the school to emphasize practical

[2] Preliminary Announcement of The Johns Hopkins School of Hygiene, 1919.

training to the detriment of the investigative work. With great firmness, however, Dr. Howell withstood these pressures and throughout his tenure maintained the scientific aspect of the school which has been in large part responsible for its current prestige and which set a pattern for other public health schools.

For his own department of physiological hygiene Dr. Howell had planned originally to include two sections, one dealing with the chemical aspects or nutrition, the other concerned with the relation of man to his environment and with the conduct of individual life. Because of the importance of nutrition and the eminence of Dr. McCollum, who was chosen for this work, Dr. Howell relinquished direction of this field, and a separate department for this part of the work was formed. Thus, the teaching and research under Dr. Howell's direction was organized to include the physiological effects of exercise and fatigue, of radiant energy, and of variations in the composition and physical properties of the atmosphere. Subsequently, he arranged for a course in industrial hazards and diseases. In addition to these courses which were primarily for the public health students, a course in physiology was given for the science students in the school, in which Dr. Howell participated for several years. He also gave a series of lectures in personal hygiene for all of the students. Throughout his entire tenure he conducted a journal club for physiologists in the School of Hygiene and the medical school.

In his teaching Dr. Howell preferred small informal classes, and his course in physiology was taught in a delightful manner. The students were expected to read a section in his textbook preceding the class. During the class period each student in turn would be asked to discuss some phase of the selected topic, and then Dr. Howell would explain those points which were not clear and describe the original experiments which supported the theories. Dr. Howell enjoyed the class himself, and the students looked forward with anticipation to each period even though they had to spend many hours in preparation. Dr. Howell believed that two qualities were essential for a good teacher—first, a sound knowledge of his subject, and second, an enthusiasm for it. Those who had the great privilege of attending Dr. Howell's classes and particularly those who studied in his department could testify to these qualities in him, but even more they remember the clarity of his lectures, his extensive knowledge and his keen interest in every subject which came under discussion. As a teacher perhaps his greatest attribute was his ability to make other persons think for themselves. Frequently, students leaving a conference with Dr. Howell would return to their own laboratories thinking what a bright

and original idea they had had, only to realize that in reality Dr. Howell had stimulated this idea by his questions. His tolerance and encouragement were truly outstanding.

In spite of all his administrative and teaching activities, Dr. Howell found time to continue his research. In line with his concept of hygiene, basic research in physiology seemed to him as appropriate for a faculty in a school of hygiene as in a medical school. Thus, except for one brief study of the relation of humidity to comfort, he did not engage personally in studies directly related to problems in hygiene. The work on heparin, described by Dr. Emmett Holt, had just been published before the School of Hygiene opened. It was only natural that Dr. Howell should continue these studies. He developed methods of preparing and purifying heparin and demonstrated that this anticoagulant was effective even when free of phosphorus and protein, that it existed in normal blood and was excreted in urine, and that it was a carbohydrate body. Studies on the photodynamic action of hematoporphyrine were published during this period, but most of Dr. Howell's research time was devoted to the problem of hemophilia. His devoted technician at the time was a hemophilic man who gladly contributed his blood for the experiments while the students supplied the control blood. Dr. Howell must have felt considerable disappointment when his experiments demonstrated that hemophilic blood did not differ from normal blood in the content of heparin, prothrombin and antiprothrombin, but he was able to show that the blood platelets were more resistant in hemophiliacs. After his retirement Dr. Arnold Rich invited him to continue his research in the department of pathology. During this period he demonstrated that blood platelets are formed in the lungs from the megakaryocytes. This discovery led him to renew his interest in thromboplastin, and he was engaged in the isolation of this material from lung tissue until the day before he died on February 6, 1945.

In addition to his administrative work, teaching and research, Dr. Howell found time to revise his *Textbook of Physiology* every three years during the period when he was in the School of Hygiene, the last edition (fourteenth) appearing in 1940 when he was 80 years of age. To publish alone a textbook on physiology was a tremendous task even in the twenties and thirties, but to keep up with the literature and to revise such a textbook at the age of 80 was amazing.

During this period, in addition to his scientific papers, Dr. Howell published two physiological reviews, one on inhibition and one on coagulation; two major lectures, the Pasteur Lecture on Coagulation and the Carpenter Lecture on Hemophilia; and a number of general papers

such as tributes to former associates and philosophical discussions of public health and medicine.

During all the years when he was at the School of Hygiene Dr. Howell maintained active participation in the American Physiological Society and in the International Congress of Physiologists. He attended the meeting of this latter group in Edinburgh (1923), Stockholm (1926), Boston (1929) and Italy (1932). His colleagues in the American Physiological Society showed their great respect and affection for him when they selected him to serve as president of the Congress when it met for the first time in America (1929), and he served as the American representative on the International Committee from its inception in 1923 until he resigned in 1932. In 1938 Dr. Howell, with Dr. Charles W. Greene, published the history of the American Physiological Society; his contribution covered the first 25 years.

In 1919 the Preliminary Announcement of the School of Hygiene stated one objective was "to develop adequate means for the dissemination of sound hygienic knowledge."[2] To further this objective Dr. Welch and Dr. Howell jointly founded the American Journal of Hygiene in 1921. Dr. Howell served as a consultant editor from its beginning and as chairman of the board from 1928 to 1931. However, he was concerned not only with the publication of scientific work in this field, but with the dissemination of sound hygienic information to the public. In line with this he established a course in hygiene for the school teachers of Baltimore under the auspices of the Johns Hopkins College for Teachers. Later in his life he served as Chairman of the Executive Committee of Science Service for eleven years.

During the time Dr. Howell was connected with the School of Hygiene, he received several honors: an LL.D from the University of Edinburgh, honorary membership in the Physiological Society (London) and the presidency of the Society of Naturalists. After his retirement he served as chairman of the Medical Section of the National Research Council and was honored by Dr. George Walker, who created the Finney-Howell Research Foundation for the study of cancer.

To be a member of Dr. Howell's department was a rewarding experience, not only because of the intellectual stimulation and assistance which he gave to all of his associates unstintingly but because of his thoughtfulness, friendliness, and generosity of spirit. When a famous physiologist came to see Dr. Howell, even the assistants on his staff were introduced. He never allowed his name to appear on a paper by a colleague or student unless he himself had participated actively throughout the experiments, and he never imposed his ideas on his staff or pressed them

to publish a paper. Dr. Howell treated the least important student with the same consideration he would show a colleague. The author well remembers her first interview with him. She had an appointment to inquire about admission to the school and was very fearful because of reports that women were not wanted at the Johns Hopkins University. Dr. Howell greeted her most kindly, however, and soon put her at ease. The conversation had hardly begun when a distinguished looking gentleman entered the room, and she was introduced to the president of the University. Anxious not to intrude she arose at once to leave, only to be reassured by Dr. Howell's remark, "Please be seated. Dr. Goodnow does not want to see me, he only wishes to hang up his coat."

Among the most delightful experiences enjoyed by his staff and students were the visits to Dr. and Mrs. Howell's home on Sunday afternoons. All of the students, particularly those from foreign countries, took advantage of the invitations to afternoon tea, and there was never a Sunday without a group of six or more present. At the laboratory Dr. Howell called everyone by his last name, but formality was put aside at home, and he called many of his colleagues and personal students by their first names. Here at home his students and staff became his friends, an honor cherished greatly by each and everyone so privileged.

It seems most fitting to conclude this discussion of Dr. Howell with the words of one of his colleagues and another great scientist, Dr. Edwards A. Park, who paid tribute to Dr. Howell at a dinner in his honor in 1941. Dr. Park said, "... as desires and tastes change under the influence of years, I long more and more to be like him, to possess his elevated standards, his keen social consciousness and feelings of duty toward his fellow men, to be able to look out on life with the wideness and clearness of his perspective, to have his lucidity of mind and the power to approach each question with such clearness, personal detachment and fairness and, finally, to possess his calm philosophy and his ability to live in the light of it. These qualities so perfectly developed and joined together appeal to me as representing the grandest kind of achievement in life and as being in their essence the highest and best accomplishments that any human being could ever seek."[3]

[3] Bulletin of The Johns Hopkins Hospital, **68**: 304, 308, 1941.

THE COURSE OF BRONCHIECTASIS IN CHILDHOOD[1]

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The course of bronchiectasis in childhood, reviewed extensively in the past (1 to 5), deserves reinvestigation in the light of the extensive use of antibiotics and resection in treatment. Improved methods for the diagnosis of cystic fibrosis, to which bronchiectasis is often secondary, permit separation of this rather special group. Bronchiectasis due to causes other than cystic fibrosis is not a common condition in childhood; however, retrospective studies on bronchiectasis in adults suggest that the symptoms often date from childhood (6, 7) and that early recognition and management are important. The course of bronchiectasis, not associated with cystic fibrosis, in children seen at The Harriet Lane Home of The Johns Hopkins Hospital since 1940 is presented in this report.

Recognition that alteration in the bronchial caliber may be associated with suppurative lung disease is attributed to Laennec in 1819 (8). The discovery, in 1918, that bronchial caliber could be evaluated by radiography after the insufflation of bismuth powder (9), and later the use of iodized oil as a contrast medium (10), made possible more accurate diagnosis of the condition during life. It was soon realized that structural changes might result from different mechanisms. Experimental studies in animals established the role of bronchial obstruction (11). Recognition that symptoms often date from aspiration of a foreign body provided clinical support for this mechanism. Since symptoms in many patients date from pneumonia, pertussis, or other inflammatory lesions, it has been postulated that the inflammation, necrosis, and scarring of the bronchial walls which occur in these infections bring about bronchial deformation (12). Deficient cartilage in the bronchial tree, a developmental defect, presumably weakening the walls of the airways, has also been invoked in the pathogenesis of generalized bronchiectasis (13). Medlar has suggested that bronchiectasis may be a developmental abnormality with infection occurring secondarily (14). He found that all ectatic bronchi do not lack smooth muscle, a finding which is usually cited as evidence of inflammatory destruction of the bronchial wall. He further pointed out that a necrotizing bronchitis would be expected to destroy the epithelial lining of bronchi as well as the re-

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mainder of the walls and that healing of necrotic areas usually is accomplished by the formation of metaplastic epithelium over granulation tissue. The epithelium of ectatic bronchi in his series was frequently normal. On the other hand, more recent experimental studies suggest that in dogs, at least, normal epithelium may recover a merseline window inserted in the trachea if inflammatory tissue is present (15). Proof of the primary event in the pathogenesis of bronchiectasis is often elusive since inflammatory changes may obscure an underlying anatomical defect.

Changing concepts in the pathogenesis of bronchiectasis may be illustrated by a medical note written by Doctor Edwards Park after a conversation with Doctor Charles Austrian about a 12-year-old white male, seen in The Harriet Lane Home on December 15, 1930.

"The child had bronchiectasis very severely. Doctor Austrian immediately examined him to see if sinus disease was present. He advised the treatment of the sinuses and postural drainage. Then he advised lipiodol treatment of the case in order to determine the nature of the bronchiectasis because the cylindrical variety was less favorably affected by section of the phrenic nerve than the saccular variety. Following the lipiodol study he advised bronchoscopy again in order to obtain secretion and determine if spirochetes were present. If spirochetes were present, he advised the introduction of neoarsphenamine into the bronchus. Finally, he thought the child would be benefitted by resection of the phrenic nerve on the left side since the pathological process on that side predominated. The interest in Doctor Austrian's consideration of the case lay in his formulation of a perfectly definite system of treatment for a condition which had seemed to me to be hopeless."

Thirty years later, with antibiotics and surgery at hand, and with much more experience with bronchography, no physician would assume that bronchiectasis in childhood is a hopeless condition.

Selection of Patients

All available hospital records from 1940 to 1960 of bronchiectasis in children under 14 years of age at the time of the onset of chest symptoms were reviewed, and as many patients as possible were re-examined in 1960. Since many of these patients were referred to this hospital from a distance, often to the surgeons directly, this series is weighted with bronchiectasis which was treated operatively. The diagnosis of bronchiectasis depended on the bronchographic demonstration of pathologic alterations in bronchial caliber, or, in one patient without bronchography, on examination of the resected lobe. Persistent atelectasis, with crowding of the bronchi, was not considered to be bronchiectasis. Patients with

chronic bronchitis and persistent localized physical signs, in many instances clinically indistinguishable from bronchiectasis, were not included. The diagnosis of cystic fibrosis was excluded in one-third of the total group on the basis of a normal sweat chloride or adequate duodenal enzymes. In the remainder the diagnosis was excluded on the basis of definite evidence of either marked improvement over many years of follow-up, the absence of nutritional deficiencies, or the presence of a clear history of antecedent illness such as tuberculosis or aspiration. It is still possible that some of these patients have cystic fibrosis of the pancreas and that their bronchiectasis is on this basis, although on clinical grounds it is improbable.

The clinical status of the patients on follow-up is described as asymptomatic, improved, no change, or worse. The presence of a chronic cough, repeated respiratory infections, rales, the need for antibiotics, and general well-being were taken into consideration. Evaluation of the patient's condition depended on his own assessment in large part, and on objective signs if present.

Environment and Family History

The group as a whole consisted of 66 patients, 54 white, 12 colored, with a slight preponderance of females, 36, to 30 males. Just under half of this group lived in the city of Baltimore. About one-third lived in rural areas. Proven bronchiectasis was not found in siblings; members of the families had undiagnosed chronic lung disease in 5 instances, tuberculosis in 12, and asthma in 7. Environmental or family status does not appear to have been associated with the incidence of bronchiectasis in this series except for the association of tuberculosis or asthma. This observation sets these patients apart from the group with cystic fibrosis in whom a family history of the disease is present in about 30 per cent (16). The incidence of prematurity was not significant since only 10 per cent of the group weighed less than 5 pounds at birth.

Onset

The onset of symptoms occurred before the age of 5 years in 74 per cent of the patients and in the first year of life in 35 per cent. Similarly, in 35 per cent of the patients in Field's series symptoms were noted in the first year of life. The usual onset was with an acute febrile illness, diagnosed as pneumonia. In more than one-third of the patients the chronic illness was attributed to the preceding pneumonia. The insidious development of a chronic cough was noted in about one-sixth of the patients. A specific statement that measles or pertussis preceded the symptoms was unusual. The illness was classified as congenital if respiratory difficulty was recalled

TABLE I

Precipitating Illness Versus Age of Onset

	Age in Years					
	<1 yr.	1–<3	3–<5	5–<10	10–<14	Totals
Pneumonia	8	5	1	4	4	22
Insidious	5	1	5	1	0	12
Pertussis	3	2	3	2	0	10
Aspiration	2	1	2	1	1	7
Tuberculosis	2	2	0	3	0	7
Measles	0	3	0	1	0	4
Congenital	2	0	0	0	0	2
Asthma	1	1	0	0	0	2
	23	15	11	12	5	66

in the neonatal period, and pulmonary symptoms dated from this time. Clearly these cases might just as well have been classified as resulting from aspiration, and similarly many of those with extensive disease and an insidious onset could have been labelled as having a congenital defect. Table I presents the antecedent illness as it was stated in the medical record. Qualifications as to the accuracy of such information should be kept in mind.

The role of endobronchial tuberculosis in the pathogenesis of bronchiectasis has been reported from this clinic (17). In the special study of the course of tuberculosis in childhood, 35 patients with bronchial obstruction were found, among whom 20 had bronchographic evidence of bronchiectasis. The onset of symptoms of bronchiectasis varied from nine months to five and one-half years after the diagnosis of tuberculosis, and in most instances was related to secondary pyogenic infection in bronchi presumably altered by the previous tuberculous process.

The most severe disease was noted in those patients whose symptoms began at less than three years of age. The prognosis was good in the group with onset after three years (Table II). All of the deaths occurred in those with early onset; however, even in this group, the number of patients who improved was greater than those not improved. Franklin (5) and Ford (2) have reported similar findings and have noted the correlation of poor prognosis with early onset of symptoms.

Linear Growth

The effect of bronchiectasis on linear growth was not striking in this group of patients. Only 13 of the 66 children were below the 25th percentile in height during their illness. Seven of the 13 had bilateral disease.

TABLE II

Age of Onset Versus Prognosis

	Age in years					
	<1 yr.	1–<3	3–<5	5–<10	10–<14	Totals
Improvement	17	5	9	9	3	43
No change	5	3	1	1	2	12
Worse	—	—	—	1	—	1
Died	—	4	—	—	—	4
Lost to f. up	1	3	1	1	—	6
	—	—	—	—	—	—
	23	15	11	12	5	66

However, 14 of the remaining 53 children in whom growth was not impaired also had bilateral disease. The only two with severe growth retardation had asthma. Recurrent pneumonia did not appear to correlate with growth retardation; 27 per cent of the undersized youngsters, and 53 per cent of those of normal stature had had more than one episode of pneumonia. The tendency of children with bronchiectasis to approach expected height stands in contrast to those with bronchiectasis and cystic fibrosis who are frequently undersized.

Sinusitis

The association of sinusitis and bronchiectasis, almost a clinical axiom, is confirmed in this study in which at least 45 per cent of the patients had radiological and clinical evidence of sinus infection. Not all records were explicit with regard to this association, so that the 45 per cent is a minimum estimate. Diamond and Van Loon noted sinusitis in 64 per cent of their patients with bronchiectasis (1). Strang (4) and Clerf (18) have emphasized the association of bilateral bronchiectasis with sinusitis. This association is not outstanding in the present study. Fifty per cent of the patients with sinusitis had bilateral disease; 60 per cent of those without sinusitis had bilateral disease. Diamond and Van Loon also report no correlation between the extent of bronchiectasis and sinusitis (1).

The presence of sinusitis did not appear to influence the prognosis of the bronchiectasis, since sixty per cent of those with sinusitis were improved with treatment and a similar fraction of those without sinusitis also improved. Three times as many patients with sinusitis had a persistent chronic cough as compared with those without sinusitis. The presence of sinusitis does appear to contribute to overall morbidity, although there is no evidence in our study that it contributes to the extent or perpetuation of bronchiectasis.

Asthma and Other Allergies

The possibility that repeated sensitization of the respiratory mucosa to allergens in susceptible individuals might predispose to structural alterations of the bronchi appears to be an unusual occurrence. Diamond and Van Loon found allergic manifestations more common in the group with tracheobronchitis than those with bronchiectasis (1), although recent observations by Van Loon suggest that allergy does play a role in many patients with bronchiectasis (19). An association of allergic manifestations and bronchiectasis has been noted in adults (20) and in children whose bronchiectasis is secondary to cystic fibrosis of the pancreas (16).

In this group of 66 children with bronchiectasis, asthma was present in 12, and associated with the onset of a chronic cough in 2. Two patients had had eczema as infants, and only one had typical hay fever. Eight of the 12 with asthma had improved on follow-up although asthmatic attacks persisted. Only 3 of the 8 asthmatic youngsters whose bronchiectasis improved were treated by lobectomy. Two of the children with bronchiectasis occurring in atelectatic lobes, secondary to asthma, subsequently had normal bronchograms. In one, bronchiectasis was demonstrated in December 1955, and had resolved by April, 1956 (Figs. 1, 2). In another,

FIG. 1. 3-year-old child during an episode of pneumonia superimposed on asthmatic bronchitis. Note dilatation of the left lower lobe bronchi.

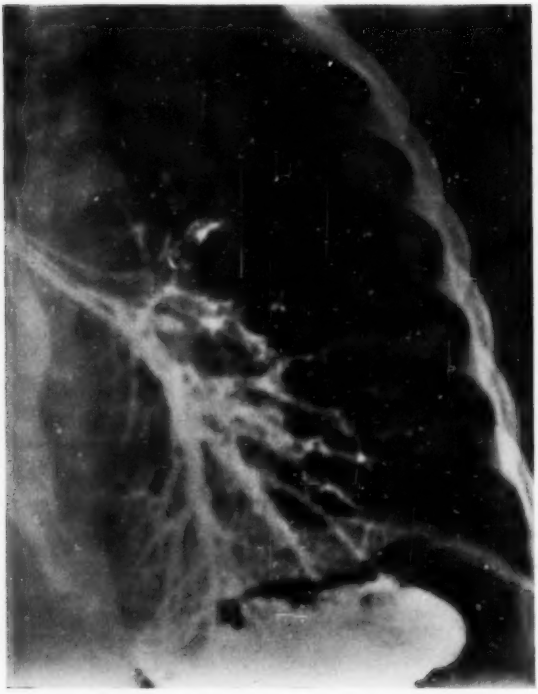

FIG. 2. Same child, 4 months after the acute episode with normal left lower lobe.

cylindrical dilatation of the right lower lobe bronchi in November 1955 cleared by April 1956. Re-expansion of atelectatic lobes with bronchiectasis has been reported (4, 21). In one study of the bronchographic changes in pneumonia, 11 of 16 patients with changes during the acute phase were normal by 4 months (22).

Prognosis after Resection

The argument for resection of localized areas depends on the evidence for the irreversible nature of bronchiectatic changes, the predisposition to chronic suppurative disease, and the threat to uninvolved lung imposed by localized infection.

The irreversible nature of saccular changes is supported by this study. While minimal cylindrical dilatation may reverse, as is illustrated in the two patients in this series, extensive cylindrical involvement and saccular dilatations were not found to be reversible in the few patients who had serial bronchograms. Clinical evidence of persistent symptoms in others

TABLE III

Follow Up (2 or More Years)

	Total	Asympt.	Sympt. or Signs but Improved	No Change	Worse	Died
Pts. with all diseased areas removed	30	19	8	2		1
Pts. with partial removal of diseased areas	16	2	10	3		1
Pts. with no resection	7	2	4 (3 adv. dis.)		1	

for months after the initial bronchogram suggested that at least saccular disease did not regress.

The problem of the threat to uninvolved lung of persistent localized disease could best be evaluated in the four patients in whom aspiration of a foreign body precipitated the localized bronchiectasis. Spread to normal lung did not occur over periods as long as one year after the aspiration. Similarly, in those patients whose bronchiectasis was secondary to endobronchial tuberculosis, spread to normal lung was not observed. Removal of the involved lobe was uniformly successful.

Thirty patients, in whom all areas diseased on bronchogram were removed, were followed more than two years postoperatively. Only 19 were asymptomatic, and 8 improved on follow-up (Table III). The persistence of symptoms in 11 patients, and later bronchographic demonstration of disease in lobes thought to be normal at the time of operation suggests that the predisposition to bronchiectasis may have been generalized in these patients, and eradication of all involved areas at one time is not a guarantee that other portions of the lung will remain healthy (Figs. 3, 4).

Bronchographic assessment of the whole lung, while essential, may not be adequate as a preoperative index of the extent of the disease. Strang's measurements of the forced expiratory volume in 39 patients with bronchiectasis revealed, in some, impairment which could be accounted for only on the basis of diffuse obstruction of the airways despite bronchographic demonstration of a localized bronchiectatic process (23).

There were 16 patients in whom only parts of the diseased areas were removed, and only 2 were asymptomatic on follow-up (Table III). Ten were improved after resection of the most extensively involved areas. In a review of 240 patients treated by operation, Streete and Salyer report poor results after unilateral resection in the face of bilateral disease (24). They believe that operation is not indicated in the face of bilateral disease, particularly of the cylindrical type. Our experience suggests that indeed the outlook is less favorable in those with bilateral disease, but evidence

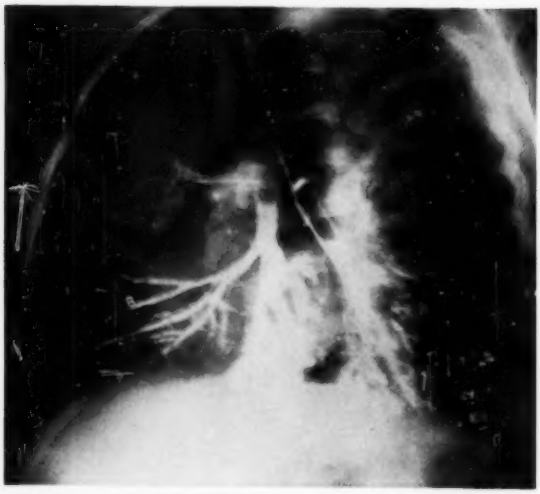

FIG. 3. Bilateral lower lobe bronchiectasis at age 7 years. The right middle lobe bronchi are normal.

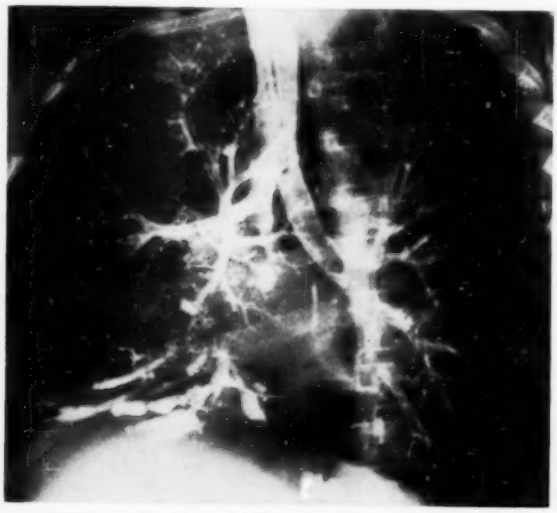

FIG. 4. Same patient 3 years after right lower lobe resection and 1 year after left lower lobe resection with advanced disease in right middle lobe.

of improvement in more than half who had only partial resections would suggest resection may have a role in the management of these patients. Rarely minimal changes on the unoperated side may regress after operation, although this might also occur without surgery.

Of a total of 57 patients who had resection, (3 at other hospitals), 2 died on the operating table, one at age 17 years in 1945, one at age 5 years in 1943. Both must be considered operative deaths. The remaining 2 deaths occurred years after operation. One patient died at age 14 years, 4 years after her second lobectomy with widespread bronchiectasis and hypogammaglobulinemia; the other died at age 12 years from disseminated tuberculosis, 9 years after right pneumonectomy for extensive saccular bronchiectasis involving all lobes on the right side.

Four patients who had extensive bilateral resections were available for long term follow-up. All four are much improved after surgery with respect to the quantity of sputum and general well being. None is entirely well, however, and all are limited to some extent in their exercise tolerance. Their course is outlined in Figure 5.

In this group of patients there were 2 operative deaths among 57 patients. In Gross' series from 1938 through 1950 there were 6 deaths among 62 operated patients, but he adds that since 1942 there have been no fatalities among 54 lobectomies on children (28).

The question of optimal timing of lobectomy in childhood with respect to the ability of the remaining lung to compensate remains obscure. Only 10 patients in this series had lobectomies by age 5 years or under, the youngest at one year of age. In four children with extensive resection before age 10 years, enough symptoms of bronchiectasis persist to cast doubt on the normality of the remaining lung. In only one was the vital capacity measured over a period of time (Fig. 6). The remaining two upper lobes grew at a rate commensurate with linear growth, and the ratio of functional residual capacity to total lung capacity was normal. He had mild lower respiratory obstructive disease on the basis of a prolonged timed vital capacity, (60% of the total expired in the first second) with improvement

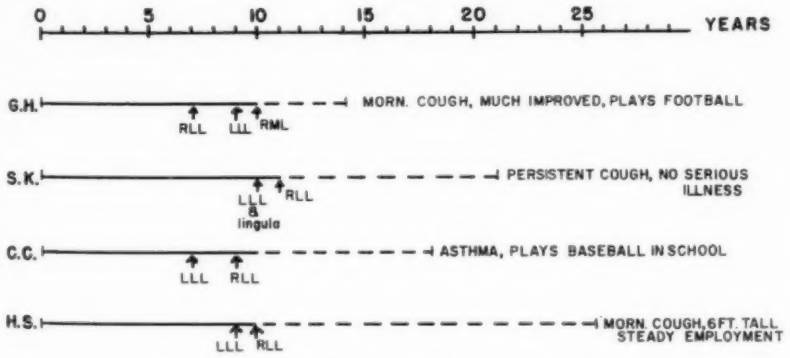

Fig. 5. Course of patients with bilateral resections.

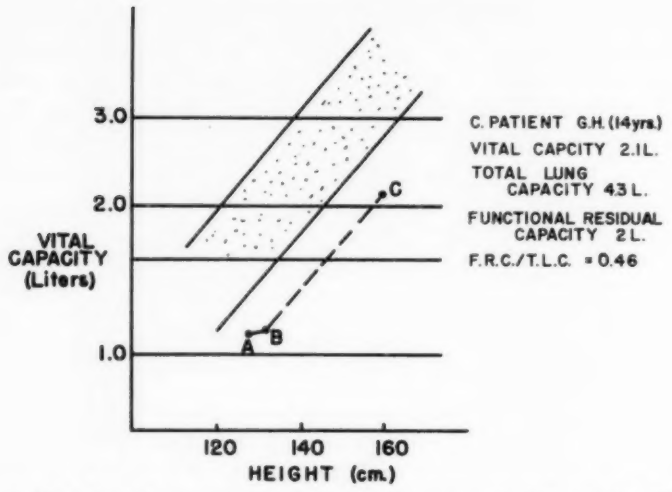

FIG. 6. The stippled zone represents the normal range of vital capacity (Helliesen, P. J., *et al.* Pediatrics, 1958, 22: 80). The increase in vital capacity in the patient after resection of lower lobes (A and B) parallels the expected increase.

after the administration of isoproterenol hydrochloride by nebulizer. If the remaining lung were surrounded by a more negative pressure as a result of lobectomy, the volume of the bronchi would be expected to increase in approximately the same ratio as that of the alveoli (25). The enlargement of the small airways would then facilitate expiration. The observation that an obstructive component exists after extensive resection suggests at least three possibilities. An underlying defect in the bronchial cartilage, as described by Williams and Campbell could involve all lobes. Bronchitis, low grade bronchiectasis, or asthma with persistent secretions may be present. Or perhaps the mechanical derangement resulting from distention of the upper lobes could distort the remaining bronchi. This phenomenon has been seen in the larger bronchi, especially in the lingula after left lower lobectomy.

Burrows *et al.* in a study of the post-pneumonectomy state in adults noted a gradual increase in vital capacity several years after operation, without any evidence of obstructive disease except in those patients who had emphysema before the resection (26). A long term follow-up of four children with pneumonectomy, reported by Cournand *et al.* (27) showed very minor deleterious effects from distention of the remaining lung. Two of the four had a significant increase in residual air and some impairment in the maximum breathing capacity. No significant pulmonary hypertension was observed. None of these patients was symptomatic at the time of study, seven to ten years after pneumonectomy.

Prognosis without Operation

Only nine patients in this group did not have resections. Seven of these have been followed for over two years, and two are asymptomatic. Three of the others had advanced disease, and of interest is the stable course. Only one is much worse, and he had bilateral saccular bronchiectasis in all lobes at the time of diagnosis. These patients as well as most of those treated operatively received courses of broad-spectrum antibiotics, some for several years continuously, others with acute pneumonic episodes only. Our impression is that antibiotics have been very useful in combatting the acute episodes. It has not been possible to eliminate all signs of infections with long term antibiotic therapy, nor has such therapy always prevented intercurrent infections.

Pathology

The pathologists' descriptions based on two or three areas from each of the resected lobes of 26 of these patients, were reviewed in the light of Medlar's observations. In 19 the condition of the bronchial epithelium was described. It was intact in 9. Specimens from 5 other patients showed only focal areas of necrosis or scarring. The bronchial epithelium was noted to be largely destroyed or replaced by stratified epithelium in only 5 of the 19 patients. Specimens from 7 patients were noted to have reduction of smooth muscle elements in the bronchial walls. One of these showed no evidence of bronchiectasis on pathologic examination although this condition had been demonstrated on the bronchogram made prior to operation. An abnormal increase in the amount of smooth muscle in the bronchial wall was noted in one patient.

COMMENT

Consideration of these patients has led us to feel that in some instances a congenital predisposition to bronchiectasis exists, perhaps in a deficiency of the bronchial supporting structures, in the nature of the secretions, in abnormal ciliary motion, or in a combination of these factors. In support of this concept we submit the following: 1. The onset of symptoms is frequently in the early years. 2. Bronchiectasis may develop in other portions of the lung months or years after removal of the original focus. 3. The presence of asymptomatic cysts is well-known; by analogy, asymptomatic bronchiectasis may be long-standing, and brought to attention by superimposed infection.

There is seldom reason for haste in recommending resection in patients with bronchiectasis. The presence of a bronchiectatic focus, if it results from aspiration or endobronchial tuberculosis does not appear to jeopardize normal lung. Since tubular disease at least may be reversible,

serial bronchograms are in order. Definite indications for surgery are irreversible bronchial obstruction or the presence of localized infections which cannot be controlled by antibiotics.

There is no evidence that lobectomy in early childhood will result in better function than lobectomy in later childhood. Indeed pulmonary reserve is such that function is always adequate after removal of a single lobe no matter what the age, and usually excellent even after pneumonectomy at any age in childhood. The four patients with extensive bilateral resections are all leading useful lives, although exercise tolerance is definitely reduced. The data are insufficient to answer the question of hyperplasia of the remaining lung after extensive resection in early childhood. The studies of Longacre and Johansmann (29) in puppies, and Bremer (30) in kittens suggest that actual generation of new alveoli may be stimulated by removal of a lung. We know of no documentation of this phenomenon in children; the one child in our series who had extensive resections at age 7, 9, and 10 years has not demonstrated a relative increase in vital capacity nor a change in functional residual capacity over the subsequent 4 years. The patients followed by Cournand *et al.* had vital capacities only slightly greater than predicted for one lung, and this may have resulted from distention of the remaining lung since the same phenomenon has been shown in adults (Burrows). The question of generation of new lung after lobectomy in early childhood remains open.

SUMMARY

Bronchiectasis diagnosed during childhood is most frequently symptomatic in the first three years of life. The presenting illness is usually an acute febrile episode diagnosed as pneumonia. The onset may be insidious, or date from an insult such as aspiration of a foreign body, pertussis, measles, or endobronchial tuberculosis.

Fifty-seven of the 66 patients in this series were treated by resection of the diseased lobes. When all the diseased areas were removed, 87 per cent were improved or well. When part of the affected areas was not removed, 73 per cent were improved or well.

The group treated medically alone is not strictly comparable to that treated by resection since occasionally operation was not recommended because the disease was very mild or very severe. Of 7 patients followed without operation, 6 were improved or well, including 3 who had advanced disease.

Spread of localized disease to normal lung in the children in whom the bronchiectasis was secondary to aspiration of a foreign body did not occur. In other children in whom the disease was due to other causes, extension

to uninvolved segments occasionally occurred even after resection of all known diseased areas.

Lung function was uniformly good after lobectomy. In four patients with bilateral resections, function was impaired but not to the extent of interfering with normal activity. Lung growth as measured by vital capacity in one patient with bilateral resection continued commensurate with linear growth, but not in excess of it.

The possibility of a congenital predisposition to bronchiectasis in some patients is discussed.

ACKNOWLEDGEMENTS

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A MODEL OF A POORLY-PERMEABLE MEMBRANE AS AN ALTERNATIVE TO THE CARRIER HYPOTHESIS OF CELL MEMBRANE PENETRATION[1]

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Among the means by which certain substances pass through cell membranes, one hypothesis proposes the existence of carrier molecules (1 to 6). The transported substance, it is speculated, combines with the carrier and the complex either diffuses through the membrane or swings, as though hinged, from one side of the membrane to the other. In either case, it is proposed that once the complex, or its business end, reaches the other side of the membrane, it dissociates, releasing the transported substance.

The data which have been interpreted as requiring postulation of a carrier system are of three kinds: First, specificity of penetration of the membrane. The fact that some molecules penetrate membranes more readily than do closely related molecules has suggested that there must be stereospecific combinations between the penetrating molecules and a carrier. Secondly, saturation. A point is reached beyond which the rate of penetration of the membrane is constant despite increases in concentration of the penetrating substance. Data of this sort have been interpreted as indicating that there is a limiting quantity of carrier. Once all carrier molecules are occupied, the transport rate is maximum. Thirdly, competition. When two related molecules, both of which can penetrate the membrane, are present simultaneously, the rates at which they penetrate the membrane are less than if either were present alone in the same concentration in which it is present in the solution of the two molecules. These data have suggested, again, that there is a finite quantity of carrier, any molecule of which can transport either substance but not both simultaneously. The second and third of these observations, leading to notions of saturation and competition, are obviously based on similar kinetic data.

The question arises as to whether there may be an alternative physical model compatible with those observations which have led to postulation of a carrier. It is the purpose of this note to propose such an alternative.

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The rates at which water-soluble non-gaseous solutes penetrate mammalian skeletal muscle membrane are very small compared to rates of diffusion of these solutes through the same unit area of a layer of water. It has been estimated that, from the flux of potassium and of aldolase, an aqueous phase of the same path length as mammalian skeletal muscle membrane would need to have only 10^{-8} of the area of muscle membrane to support the observed fluxes (7).

It is, therefore, appropriate to examine the properties of a system in which the number of apertures in the membrane are small compared to the number of molecules capable of diffusing through the apertures. If P is the probability, in the absence of interaction among molecules, that any given molecule will penetrate the membrane during the time between t and $t + dt$, then, if there were no interaction among molecules, the flux, ϕ, through the membrane from the side in which the concentration of molecules is n will be nP. However, because only a finite number of molecules can occupy the apertures in the membrane during the time between t and $t + dt$, it can be anticipated that as n is allowed to increase there will be impacts between molecules in the vicinity of the apertures and that these impacts will have the effect of diminishing the probability that one or more of the colliding molecules will penetrate the membrane during the interval between t and $t + dt$. The flux will, therefore, be $\phi = nP - \theta(n)$, where $\theta(n)$ describes the number of impacts which diminish the probability of penetration.

This simple relation ignores the role of solute. That is, the model which we are about to examine is obviously appropriate to gases, but its relevance to solutions remains to be demonstrated.

The problem which we pose does not seem to have been examined by those interested in kinetic theory of gases. Although there have been studies of effusion of gases through small holes, a process called transpiration when true molecular streaming occurs, the holes have been very large compared to the size of the molecules. In general, the study of molecular effusion has been directed toward testing certain hypotheses of kinetic theory and for this purpose the experimenter has been concerned primarily with the relation between the diameter of the hole and the mean free path of the molecules of the gas. For most gases at atmospheric pressure and at about 15°C, the mean free path is about 100 times the diameter of the equivalent sphere of the gas molecule. The holes employed experimentally have been of the order of tens of microns in diameter and the mean free path has been increased by operating at low pressures. The assumption, in these studies, that collisions between molecules are negligible, is probably correct, but the model is not applicable to our present study.

Some properties of P may be developed from classical considerations of

a maxwellian gas. The probability of a molecule's penetrating an aperture in the wall of a container can be derived from the classical problem of the molecular beam in which molecules of one sort are permitted to stream through molecules of a second sort. The probability per unit time of a collison per molecule of the first sort with any molecule of the second sort (see anv standard text on kinetic theory of gases) is

$$P = mS(\bar{v}_1^2 + \bar{v}_2^2)^{1/2}/V, \tag{1}$$

where m is the number of molecules of the second sort, S is the cross-sectional area of the mutual sphere of influence of the two types of molecules, $\bar{v}_1$ and $\bar{v}_2$ are the mean velocity of the first and second type, respectively, and V is the volume of the container. For the case of molecules striking an aperture, m is the number of apertures and $\bar{v}_2$ is zero. If the apertures have radius r, then to penetrate the apertures, spheres of radius s must enter an aperture at a distance never greater than $(r - s)$ from the center of the aperture; that is, the penetrating sphere must collide with an imaginary sphere of radius $(r - 2s)$ concentric with an aperture. The radius of the mutual sphere of influence of colliding molecules is the sum of the radii of the two molecules. In this case it is $(r - 2s) + s = r - s$. Therefore, for the case of molecules penetrating apertures,

$$P = \pi \bar{v} m(r - s)^2/V,$$

and for n molecules the probability of penetration in the absence of interaction between molecules is

$$nP = \pi \bar{v} mn(r - s)^2/V. \tag{2}$$

Unfortunately there are many uncertainties about the structure of $\theta(n)$. It is, of course, easy to formulate the probabilities of collisions between two molecules in the volume, V. From eq. (1), the probability of such collisions per unit time is

$$nP_n = n^2 \pi (2s)^2 \cdot \sqrt{2} \cdot \bar{v}/V. \tag{3}$$

We are, however, not interested in every collision between molecules but only in those collisions that diminish the probability of penetration. Specifically we demand that, to be included in $\theta(n)$, a molecule just about to enter the aperture collide so that, whereas two molecules would have penetrated the membrane only one or none does, or if only one of the colliding pair was otherwise destined to penetrate the membrane it does not. P_n is therefore too large. The model requires that there be some critical volume about each aperture in which anti-penetrating collisions occur. The size of this volume is uncertain, but it must be a function of the radius of apertures, the radius of molecules and their velocity, and it may also be a function of

the molecular density. We shall designate this volume by $f(r, s, \bar{v}, n)$. There will be m such volumes and they constitute a fraction $mf(r, s, \bar{v}, n)/V$ of the total volume. Not every collision occurring in this critical volume will be detrimental to penetration. Presumably in consequence of some collisions molecules which were not headed for penetration may be redirected toward an aperture. Although certain assumptions might be made about the size of the critical volume and about the fraction of potent and impotent collisions occurring in it, these assumptions are so tenuous as to make further formulation profitless. Nevertheless, unless the factors by which eq. (3) must be multiplied to convert it to $\theta(n)$ are functions of n and are either exponentials in n or polynomials in powers of $(1/n)$, $\phi(n) = nP - \theta(n)$ will not behave like a saturable reaction. As it stands, unless $\theta(n)$ is such a function, $\phi(n) = nP - \alpha nP_n$, where α is not a function of n, is the equation of a parabola (of the form $y = a_1x - a_2x^2$) which rises from the origin to a maximum ϕ, falls symmetrically to zero and becomes negative for larger n.

It is owing to uncertainties about the behavior of $\theta(n)$ that we resort to examination of a physical model. Some properties of θ are, however, obvious intuitively. The probability of penetration in the absence of interaction increases with $(r - s)^2$. θ should increase less rapidly. Thus, in an impact between two spheres of radius s, one of which lies within the outer surface aperture of radius r, the second sphere may or may not lie within the surface of the aperture. It must, however, strike a circle of radius $r + s$, concentric with the aperture. The probability that the first sphere will enter the aperture at its periphery; that is, that it will enter the annulus defined by the area $\pi r^2 - \pi(r - s)^2$, if it enters the aperture at all, is $[r^2 - (r - s)^2]/(r^2) = (2rs - s^2)/(r^2)$. Therefore, the greater r, the greater the probability that impact will not occur at the periphery of the aperture. It can be assumed that collisions in the central region of the aperture will be less likely to alter the probability of penetration than those at the periphery. Therefore, as r increases, θ will not be expected to increase as much as P.

Similarly, as s increases, nP decreases with $(r - s)^2$, or $dnP/ds = constant \times [-2(r - s)]$. $\theta(n)$, however, will increase with s.

These considerations imply that if $\phi(n)$ is asymptotic, the larger r and the smaller s, the larger n must be at just-maximal value of ϕ.

The effect of altered velocity is unpredictable. From eq. (2) nP increases linearly with $\bar{v}$. Collisions between particles also increases linearly with $\bar{v}$, but because the effective collisions, as we have defined them, are confined to a volume which is an unknown function of $\bar{v}$ there is no *a priori* argument for the effect of $\bar{v}$ on $\theta(n)$.

If two species of molecules, one a sphere of radius s_1, the other a sphere of radius s_2, are introduced into the system, all other things being equal,

the combined flux of the two species, assuming that both are sufficiently small to penetrate the membrane, will be as though there were a single species of radius $(s_1 + s_2)/2$. At a concentration n_1 of s_1 and n_2 of s_2, $d\phi_r/dn$ will behave with respect to n as though there were a concentration $n_1 + n_2$ of spheres of radius $(s_1 + s_2)/2$, where ϕ_r is the combined flux. If one examines the flux of either species under these circumstances, it will be less than if either species were present alone at its concentration, because $d\phi/dn$ decreases with n.

In brief, then, a poorly-permeable system may be expected to exhibit saturation and competition.

To examine these hypotheses and to obtain data with which to elaborate further details a model was constructed.

A Lucite box was divided into two chambers by a removable Lucite sheet in which a hole was drilled. Each chamber was approximately three inches cubed and the divider was $\frac{1}{8}$ inch thick. In the lower chamber were placed steel bearing balls. In a corner of the upper chamber was fixed a magnet to serve as a particle collector. The magnetic field through the divider and in the vicinity of a hole was negligible. The assembly was mounted on a wrist action shaker. Although the motion of the shaker was not random, for purposes of the model it supplied a reasonable analog of thermal motion. The steel balls were considered analogs of molecules. The plastic divider was the cell membrane with a single break in its barrier and the magnet was a cellular concentrating mechanism which served the practical purpose of permitting only one way flow of molecules. The number of penetrations of the membrane as a function of the concentration of particles outside the cell could then be stated simply by counting the steel balls affixed to the magnet at the end of a period of shaking. Precautions were taken to assure that apparent changes in penetration were not due to saturation of the magnet or to changes in particle velocity owing to overcrowding in the lower chamber. The model, then, is an analog for a passive process. Particles passed from the lower to the upper chamber only as a result of agitation.

Figure 1 illustrates saturation. The number of penetrations approaches a constant independent of the number of steel balls in the lower chamber.

Figure 2 illustrates that saturation occurs at a lower concentration as velocity decreases and that flux increases with the velocity of the spheres.

Figure 3 illustrates that when $(r - s)^2$ is changed by changing the radius of the spheres, keeping the membrane constant, flux increases as s decreases and saturation is reached only at a greater concentration as s decreases.

Figure 4 illustrates that when $(r - s)^2$ is changed by changing the radius of the aperture through the membrane, flux increases and saturation is postponed with respect to concentration. Figure 4 suggests that the im-

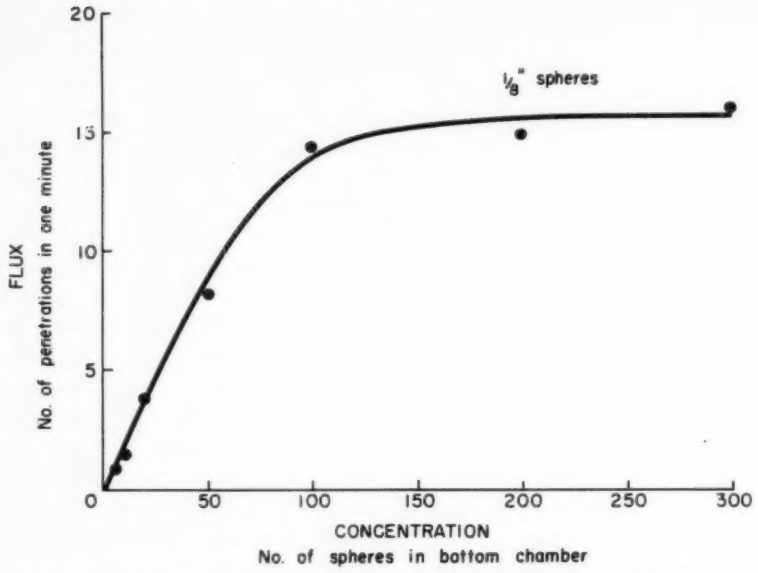


FIG. 1. "Saturation". In this and subsequent figures, except Fig. 5, each point is the mean of at least 9 trials. At low concentrations 30 trials were made.

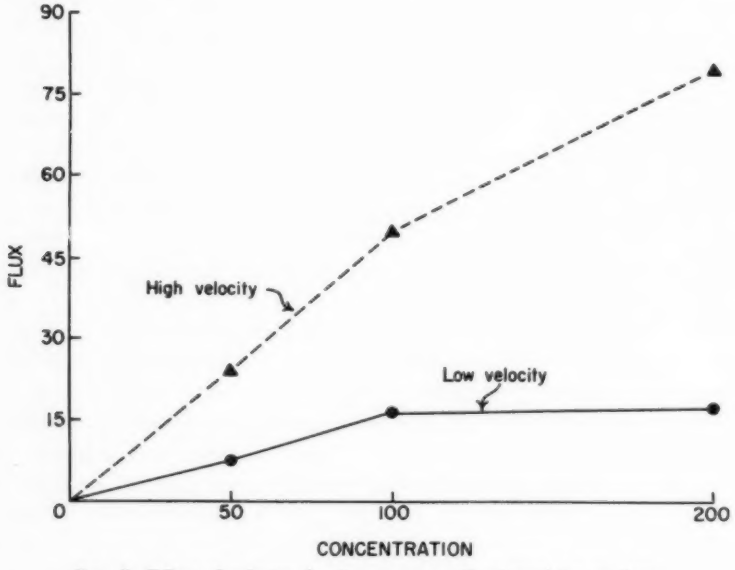


FIG. 2. Effect of velocity (= "temperature") of particles on flux.

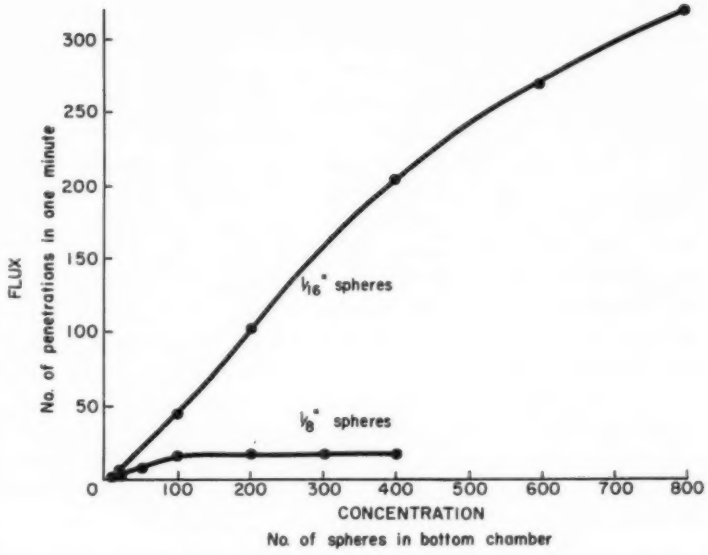


FIG. 3. Effect of particle radius on flux.

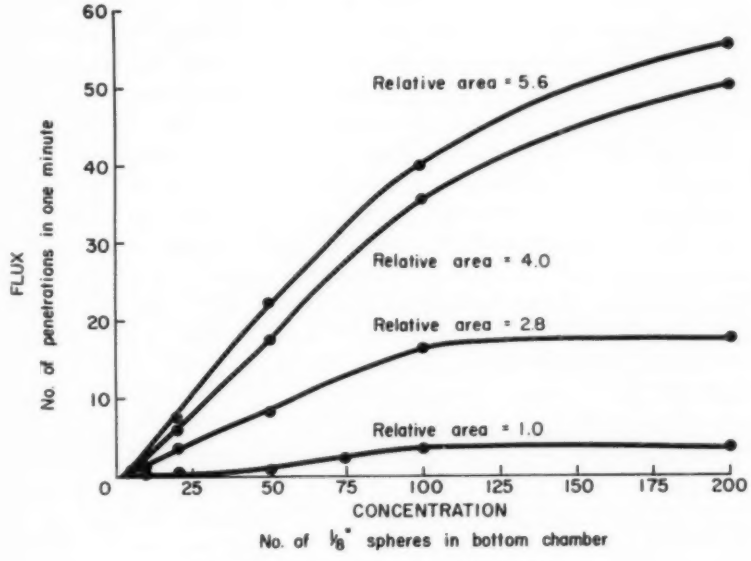


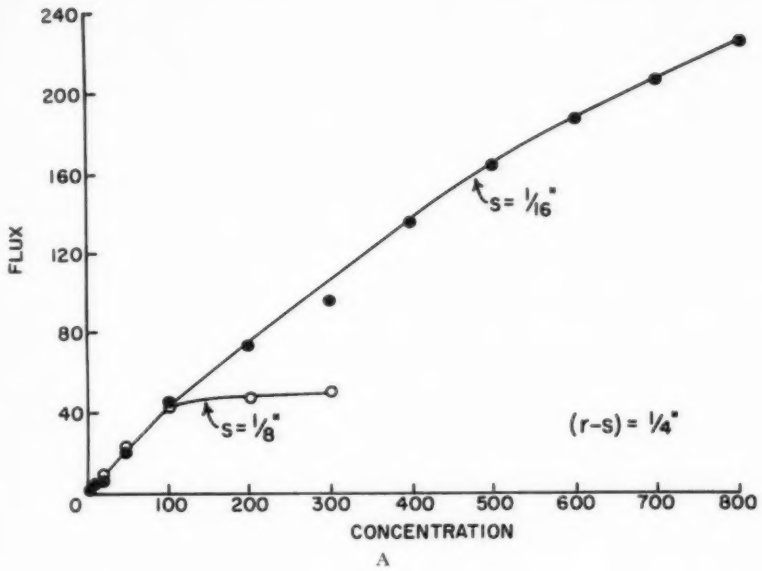
FIG. 4. Effect of aperture radius on flux.

portant collisions, i.e., those that decrease flux, occur in the vicinity of the aperture. If saturation were owing only to an increase in collisions between molecules everywhere in the chamber with consequent reduction in the mean free path of the molecular population, then saturation would be a function only of the number of particles and not of the dimensions of the aperture. In fact, however, the concentration at which saturation occurs is a function of the area of the aperture as predicted in the introduction.

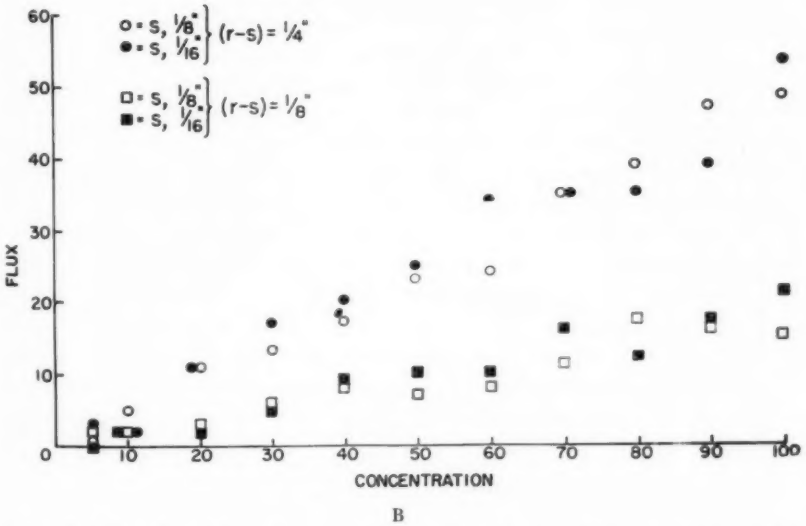
This point was examined further by replacing the membrane with a solid sheet of Lucite on the under surface of which was installed a copper wire grid so constructed that an electrical circuit was closed whenever a bearing ball struck the grid. The output was led into a scaler and the rate of impact between steel balls and the membrane was recorded as a function of increasing concentration of steel balls. The scaler was able to resolve single events occurring several hundred microseconds apart so that it is quite likely that most collisions between spheres at the membrane were recorded correctly as two hits. The impact rate increased fairly linearly with concentration over the range of concentration examined (up to 200 spheres), demonstrating that the increase in collisions between spheres which must occur with increasing concentration was unimportant in producing the phenomenon of saturation.

Eq. (2) predicts that P should be proportional to $(r - s)$. At low concentration when $\theta(n)$ may be small, flux should be proportional to $(r - s)$. With $(r - s)$ constant and s varied, flux was indeed independent of s at low concentrations (Fig. 5), confirming eq. (2) in that respect. At larger concentrations, where $\theta(n)$ became prominent, flux and, therefore $\theta(n)$, was clearly a function of s. The quantitative relation between the two experiments illustrated in Figure 5 is complicated by the fact that the thickness of the membrane, which was the same for both sets of spheres, limits the range of vectors which will permit penetration. A small sphere may take a greater angular path through the aperture, without striking the wall of the aperture, than a large sphere. Nevertheless, because flux at low concentrations was about the same, it is likely that the effect of membrane thickness was negligible.

Figures 6 and 7 illustrate competition or inhibition. When the flux of spheres of diameter $\frac{1}{8}$ inch is examined in the presence of spheres of half that diameter, it is decreased at sufficiently high concentrations whether the experiment is done by holding the concentration of $\frac{1}{8}$ inch spheres constant and varying that of the smaller spheres, or by varying the concentration of $\frac{1}{8}$ inch spheres in the presence of a constant number of smaller spheres. With small concentrations of both spheres there is a true increase in flux beyond that which occurs when only one size is present. This may be related to the fact that the curve relating flux to concentration is sig-



A



B

FIG. 5. Effect of particle radius on saturation when difference between aperture radius and particle radius, $(r - s)$, is constant. A. Over wide concentration range, showing that saturation occurs at lower concentration for larger s. B. Detail over smaller concentration range to show that flux at low concentration is a function of $(r - s)$ and not of s.

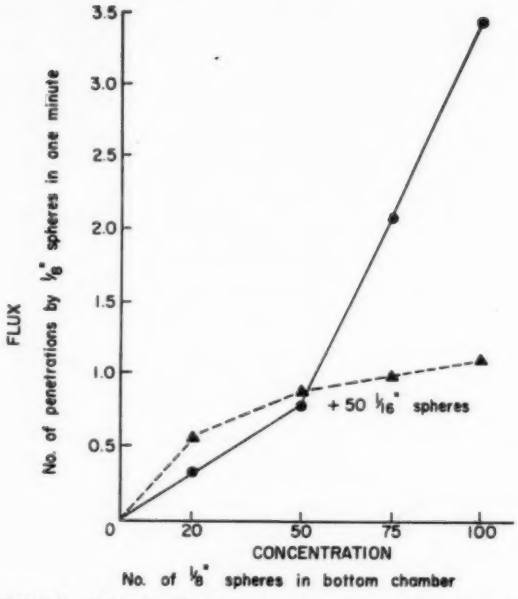


FIG. 6. "Inhibition". "Inhibitor" concentration constant; "substrate" concentration varied.

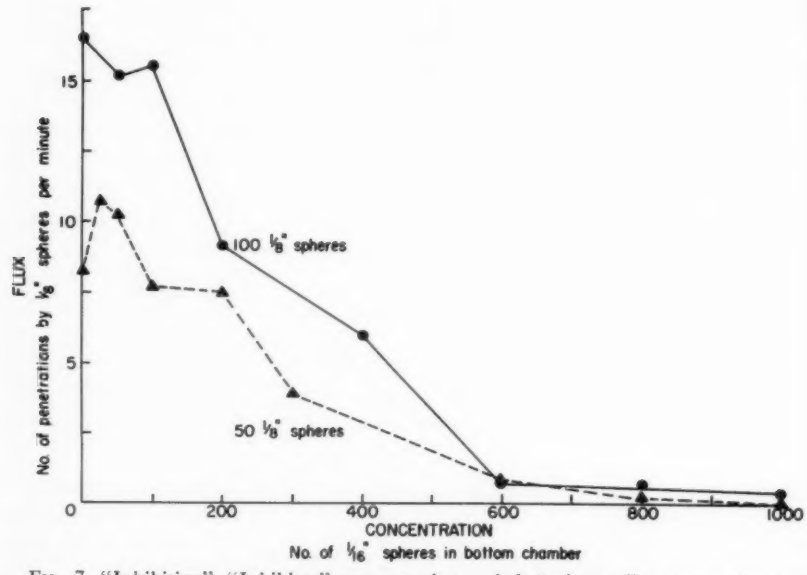


FIG. 7. "Inhibition". "Inhibitor" concentration varied; "substrate" concentration constant.

moid (see Fig. 4, for example). The net probability of flux per sphere, ϕ/n, increases with concentration, for small concentrations. Therefore, when the total number of spheres is increased by addition of a second species, ϕ/n behaves as though it is operating at a somewhat higher concentration of each of the species. It increases in the vicinity of the foot of the sigmoid curve and decreases at higher concentrations. It is possible that the foot of the sigmoid curve is caused by collisions which produce a penetration when neither sphere would have otherwise penetrated. This event would occur when one sphere was close to the periphery of the aperture. It would therefore be most frequent at the smallest aperture, in which the ratio of circumference to area of aperture is greatest, and in fact it was (see Fig. 4).

In general, then, the simple mechanical model demonstrated the validity of the hypothesis: Saturation and competition are inherent properties of a purely passive system in which the number of diffusible particles is large compared to the number of available apertures through the membrane.

The phenomenon of specificity has not been investigated but it is easy to imagine that conformational specificity might be assigned to configuration of the aperture in the membrane. This could be imitated by machining particles and apertures of specified size and shape.

The hypothesis presented here arose from problems concerning the mechanism of action of insulin and the movement of glucose from interstitial fluid into cells, particularly skeletal muscle.

Glucose moves into skeletal muscle from a solution in which its concentration, in normal mammals in the basal state, is about 5 mM to an intracellular solution in which its concentration is virtually zero. That is, glucose uptake by muscle coincides with a favorable chemical potential difference. In the face of this fact, one wonders why a carrier might be desirable from nature's point of view. The evidence for a carrier for glucose transport is indirect. It is based on observations which have been interpreted as saturation and competition (2, 4, 6, 8), phenomena which, it has now been shown, can occur in a purely passive system and it is based on a certain specificity of penetration. With respect to a cell in which insulin has no effect, the erythrocyte of man, this specificity is conformational (9) and is compatible with the notion of conformational specificity of apertures, although this is not the interpretation which has been put on it in the past.

In general, insulin seems to increase glucose uptake by those tissues in which glucose uptake is otherwise quite small. Those cells in which endogenous glucose uptake is brisk do not, in general, respond to insulin.

Skeletal muscle poses a formidable barrier to diffusion of glucose. Its permeability increases when the muscle is damaged, as in the excised, cut rat diaphragm. In the case of excised rat diaphragm, glucose uptake, in

the absence of exogenous insulin, increases with glucose concentration in the bathing solution up to about 600 mg. per 100 ml. (10). This is not true for forearm muscles of intact man. Glucose uptake by forearm muscles *in situ* is very much less than that of the rat diaphragm and cannot be increased by doubling the concentration of glucose in arterial blood supplying the forearm (11). Thus, in terms of the model described here, skeletal muscle *in situ* behaves as though it were a poorly permeable membrane exposed to a large concentration of glucose and, at glucose concentrations normal to the basal state, as though the flux of glucose were at its maximum and constant value.

In such a system, glucose influx can be accelerated only by removing competing molecules or by increasing permeability. When insulin is delivered to forearm muscles, by constant intra-arterial injection in minute concentration, there is a very large increase (about ten-fold for a local insulin concentration of 10^{-4} units per ml. of plasma) in glucose uptake (12). This is most simply interpreted, in the view adopted here, as owing to a configurational change in the membrane such that permeability to glucose is increased. This could be accomplished by increasing the number of apertures through which glucose diffuses or by increasing the area of individual apertures.

It has been shown previously that insulin hyperpolarizes rat skeletal muscle (13) and that it decreases efflux of potassium and probably also the influx of potassium but to a lesser extent (14). These observations were interpreted as secondary to a direct effect of insulin on the membrane in which the positive fixed charge of the membrane was increased. It is postulater further that the same distortion of the membrane which leads to altered membrane potential and altered potassium flux results in a change in permeability to glucose.

It has been suggested previously that there are no fixed pores, in the sense of permanent structures, through the membrane of skeletal muscle, but that breaks in the barrier to diffusion sweep across the membrane so that the system behaves during some interval of time as though it had a certain average number of apertures through which material diffuses (7). The membrane is further complicated by the fact that it appears to be double-layered. If the sweeping process in the outer protein layer is independent of that in the inner protein layer, solute particles must then spend a certain amount of time in the membrane and, in particular, between the layers of the membrane. Conformational specificity may reside in either or both layers. Increased permeability, in such a model, can be accomplished in one of three ways, or by a combination of the three: (a) by increasing the frequency with which apertures sweep the membrane, (b) by increasing the duration of time during which apertures remain open, or (c) by in-

creasing the mean area of the apertures or by altering their shape, say from a rhomboid to a square, without a change in area, so as to permit diffusion of larger molecules. For systems which have conformational specificity only (a) and (b) are permissible. Conformational specificity might be created by (c). The sweeping process is considered to be oscillation, perhaps thermal, about a stable configuration. It is perhaps simplest to conceive of insulin action as one which alters the fundamental configuration to a new stable configuration.

In addition to insulin, there are other means of increasing glucose uptake by muscle. It has been known for sometime that exercise or electrical stimulation of muscle has such an effect (15) and it has more recently been pointed out that anoxia and several metabolic inhibitors do also (16). In the latter case Randle and Smith (16) suggested that these data supplied evidence of a process by which glucose was extruded actively from muscle. However, electrical stimulation of muscle, depolarization of muscle, anoxia and various metabolic inhibitors (7) all make muscle more permeable to aldolase, which is interpreted as flowing from muscle simply by diffusion. It is consistent, therefore, to interpret the effect of these events as one in which permeability is increased and diffusible substances of all sorts diffuse more rapidly and in accordance with their appropriate chemical potential differences.

The general probability equation for flux, $\phi = nP - \theta(n)$, given here is deliberately noncommital concerning mechanism. It is apparent that the aperture in the membrane is an analog of either an enzyme or a carrier, both present in limiting quantity. The equation could be written, for example, in the Michaelis-Menten mass action form, $\phi = \phi_{max}\, n/(K + n)$, where K is an association constant between the spheres and the aperture, and ϕ_{max}, the constant maximum flux, is the product of some constant and a function of the fixed concentration of apertures, independent of the concentration, n, of spheres. Tests for goodness of fit of the data to this and other first order equations demonstrated a number of departures, probably owing to the non-random nature of the shaking process which imparted parabolic paths to the spheres in flight.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

April 10, 1961

PHILOSOPHY OF PELVIC SURGERY

R. W. TeLinde

Surgical technic is important but perhaps even more important is the philosophy of the surgeon. There is much unnecessary pelvic surgery done which does no good and often is harmful. There is incomplete surgery which may not cure a malignant lesion and prevent its cure when adequate surgery is attempted later. And there is ultra-radical surgery done on hopeless cases which only adds misery to the patient's terminal days.

As students, the elder Dr. Finney used to tell us there were three indications for surgery: 1) to save life, 2) to relieve suffering and 3) to correct deformity. If one cannot justify his contemplated surgery on the basis of one of the above three indications, he should take a long look at the situation before proceeding.

The various surgical lesions of the pelvis are considered in the light of the above criteria. The treatment of the benign myomata, for which unnecssary surgery is often done, is considered. It is emphasized that definite symptoms must be present to justify surgery. Congenital and acquired anatomical defects are evaluated in consideration of their correction. The problem of ovarian tumors, much like that of lumps in the breast, is appraised. Since malignancy may occur in ovarian tumors at all ages they should be considered seriously, particularly because they are silent growers. The removal of asymptomatic ovarian growths is mandatory, even though the pelvic examination may give no hint of malignancy.

Endometrial carcinoma and cervical cancer are viewed in the light of the author's experience. The place of irradiation and surgery in these two diseases is evaluated. Finally the place of ultra-radical surgery in recurrent and advanced pelvic cancer is appraised and the author's criteria for such surgery are given.

TREATMENT OF NEURAL DEAFNESS WITH PREDNISONE

Richard D. Hahn, Philip Rodin and Harriet L. Haskins

1) Nineteen patients with neural deafness due to congenital syphilis and twenty patients with neural deafness of other origin have been treated with prednisone in gradually tapering dosage. Dosage was in general regulated according to the results of standard pure tone air and bone conduction audiometry, speech hearing threshold, and discrimination score, measured before, and at short intervals following the commencement of therapy.

2) The neural deafness of congenital syphilis occurs late, often not until adult life. It is usually an isolated neurological finding without abnormalities in the cerebrospinal fluid. Its development and progression are not prevented by treponemicidal therapy, even with penicillin. It is almost always bilateral and usually of gradual onset, although there may be either sudden onset or suddent dramatic worsening. Episodes of vertigo and continuous or intermittent tinnitus are common. Hearing impairment is often

fluctuant but, in the absence of effective treatment inexorably progressive at a highly variable rate. The major difficulty is in discriminating speech.

3) Objective improvement, often marked, occurred during the first month of prednisone therapy in 10 of the 19 congenital syphilitics, and was subsequently maintained. In 3 therapy was deliberately stopped, with no retrogression over periods of 40 months, 1 month, and 2 months respectively. In the 9 without definite improvement progession may in some instances have been prevented.

These results show that the neural deafness of congenital syphilis is not usually a purely degenerative process.

4) In long-standing neural deafness of undetermined etiology prednisone therapy was in general unsuccessful.

5) In sudden non-syphilitic deafness of neural type which is often unilateral and occurs with or without an apparent concomitant upper respiratory infection, prednisone therapy sometimes results in dramatic improvement and may be urgently indicated.

EFFECT OF TETRACYCLINE ON CHRONIC BRONCHITIS

P. S. Norman, E. W. Hook, Jr., R. G. Petersdorf and L. E. Cluff

Patients with chronic bronchitis have been demonstrated to have long-standing and continuous infection of the lower bronchi with organisms that are considered normal inhabitants of the mouth and throat and 2 species have been particularly implicated in exacerbations of this disease, namely, *Hemophilus influenzae* and *Diplococcus pneumoniae*. In order to determine whether long-term treatment with a broad-spectrum antibiotic would influence the bronchial flora in chronic bronchitis or the clinical course of the disease, a 2 year controlled study of the effect of tetracycline has been done.

The patients under study had chronic non-tuberculous infection which had been accompanied by cough and sputum for at least two years and must have had occasional exacerbations with purulent sputum. Using a double-blind technique, these people were given either 1 gm. of tetracycline a day or indistinguishable placebos for a three-month period. At the end of this time without their or their physician's knowledge they were switched to the opposite drug for an additional three-month period. If they continued on the study, an additional 6 months was observed, 3 months on tetracycline and 3

TABLE I

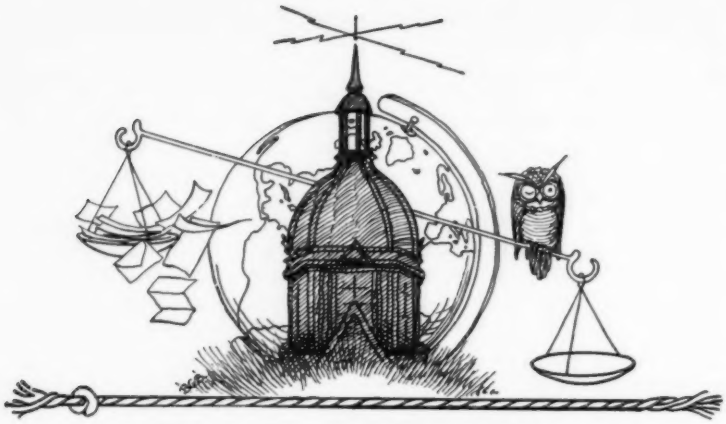
Clinical Evaluation

	Placebo Periods	Tetracycline Periods
Clinical Status		
Better	13	23
Same	32	37
Worse	28	15
Dyspnea		
Better	12	22
Same	34	38
Worse	27	15
Sputum		
Decreased	11	24
Same	37	37
Increased	25	14

months on placebo. Thus, each patient acted on his own control. During treatment the patient was seen at monthly intervals and the response to therapy was evaluated clinically with particular attention to the number and frequency of exacerbations but also to severity of cough, changes in exercise tolerance and sputum production. Sputum samples were taken for quantitative bacteriology, physical findings were recorded and pulmonary function tests were taken.

Thirty-three patients have been observed for 148 periods, 73 on placebo and 75 on tetracycline. The Table shows the blind clinical evaluation of these periods. Altogether, 45 exacerbations of the disease occurred during placebo and 19 occurred during tetracycline. At the same time 63 tetracycline periods were free of any exacerbation while only 41 placebo periods were free of exacerbation. Statistical analysis of the data indicated that the reduction of exacerbations was significant at the 2 per cent level and was highly correlated with clinical improvement.

Tetracycline resulted in a distinct lowering in the frequency of isolation of two organisms, the pneumococcus and hemophilus. This reduction was significant at less than 1 per cent and was highly correlated with reduction in numbers of exacerbations. There was not a concurrent overgrowth of antibiotic resistant organisms such as staphylococcus, gram negative organisms or fungi. These results indicate that long-term use of tetracycline in low dosage exerts a mild beneficial effect on the course of chronic bronchitis, particularly on the number of exacerbations of the disease.



BOOK REVIEWS

Principles of Human Genetics, 2nd edition. By CURT STERN. 753 pp., $9.50. W. H. Freeman & Co., San Francisco, Cal.

At last the revision of a classic awaited for over ten years has appeared. The organization and emphasis are the same as in the first edition but extensive rewriting and considerable expansion has been necessary to incorporate the rapid developments in human genetics in the last decade. Our anticipations have been adequately satisfied.

A weakness is, possibly, in the area of biochemical genetics and the chemistry of heredity. DNA or desoxyribonucleic acid does not appear in the index. In the chapter on "Genic Action" or elsewhere it would be useful to have at least a brief resumé of the current "dogma": that the genetic information is somehow coded in the sequence of purine and pyrimidine bases of DNA; that the primary function of the gene is to specify the amino-acid sequence of proteins; that RNA is somehow involved in transfer of the code from its place of storage in the chromosome to the site of primary gene action, that is, protein synthesis, in the ribosomes of the cytoplasm; that all characteristics of proteins are a logical result of the amino acid sequence. The studies of human hemoglobin are to a large extent responsible for development of the modern dogma and justify inclusion in a textbook of human genetics.

Nevertheless, Stern's book is the best single text we have to recommend to the student of human genetics.

VICTOR A. MCKUSICK

Basic Pharmacology for Nurses, 2nd edition. By JESSIE E. SQUIRE. 275 pp., $3.50. The C. V. Mosby Co., St. Louis, Mo., 1961.

This is a soft cover book dealing with basic pharmacology presented in a very simple format. Holes have been punched through the entire book so that any or all pages can be removed and inserted into a two or three ring binder. Essentially this book is divided into three parts.

Part I deals with general information regarding drugs and their dosage, i.e., drug standards, legislation, sources, preparations, administration of medicines and computations of various dosage, using both the apothecaries' and the metric system. The procedure for administration of medicines is listed step by step.

Part II lists a limited number of drugs, giving the pronunciation for each in parenthesis, giving the use of the drug and toxic signs and symptoms. There is included, also, a brief statement regarding indications for use of the general category of drugs.

Part III contains specific assignments designed to review the material in each chapter or unit of the text.

Since much material is covered in such a limited space, the information tends to be broad but superficial thus losing much of its value as a textbook for professional nurses. However, it could be valuable as a text for the teaching of practical nurses.

BARBARA HIBYAN

Allergie-Probleme im Kindesalter. By GERHART ERDMANN. 165 pp., Brosch. DM 14,60. Johann Ambrosius Barth, Leipzig, Germany, 1961.

Clinical investigations and experimental work in the field of allergy have developed in Europe on a much smaller basis than in the United States. The first journal devoted to this specialty, entitled "Allergie und Asthma", appeared in Germany only a few years ago. Several supplements to this journal have since been published, and one of them is Erdmann's monograph. It was the author's intention to give a survey of the problems and to stimulate the growth of interest in pediatric allergy which he feels was neglected in his country while in other countires excellent textbooks on this subject have appeared and special hospital departments devoted to its study have been created.

The small volume should serve its purpose well. The existing literature has been thoroughly studied and has been used freely, at times not with sufficient discrimination. It is to be hoped that a new edition will show more prominently and with more details the author's personal experiences and observations. This holds especially for schemes and results of specific desensitization, preparing extracts for skin testing and treatment, and statistical material.

Nine chapters of the work deal with hereditary factors, diagnosis, skin manifestations, serum sickness, drug allergy, immunization reactions, food allergy, hayfever, and asthma. Rheumatic fever, acute glomerulonephritis, and allergies of the urinary tract are discussed under the heading "Infectionsallergie." Migraine, chorea minor and some other neurological disorders are considered in a chapter on neuro-allergy. Principles of treatment, and a discussion of auto-antibodies in pediatrics, a field in which the author has done some original investigations, conclude the volume. The bibliography covers more than eight pages of narrow print with an extensive but somewhat uneven selection of European and American literature.

One is surprised to find in the pollination calendar of the important hayfever plants (p. 94) goldenrod listed but oak pollens missing. The statement on page 135 that antihistamine drugs and corticosteroids give gratifying results in anaphylactic shock is dangerously misleading.

Print, paper and illustrations conform to the high standard usual for German publications.

S. WALTER LANDAU

Neurology Simplified. By DAVID JOSEPH LAFIA. With a Foreword by A. Earl Walker. 175 pp., $6.75. Charles C Thomas, Springfield, Ill., 1960.

In our days of superspecialization we should always welcome any effort toward simplification. This is increasingly true in the field of neurology, which, as Dr. Walker puts it in his foreword to the book, "is regarded by most of the doctors as a mysterious subject

which they neither hope nor desire to understand." However simplification is not synonymous with elementary approach and therefore is a rather difficult task.

Dr. LaFia is evidently basing his presentation on personal experience. He emphasizes the most essential factors in common neurological disorders and talks a language which should be understood readily by the practitioner most alienated to neurology. However, it seems to this reviewer that the author underestimates the student of medicine, whose standards in neurology are higher than the author anticipates.

This little book helps to recall and straighten out old memories in a field which rapidly became extremely complicated, and in so doing it may well be of use to students and general practitioners. The first part of the book deals with techniques of neurological diagnosis from the point of view of etiology, anatomy and clinical manifestation. The second part is dedicated to an encyclopedic discussion of the commonest neurological disorders.

The presentation is clear and well-organized. It is regrettable that there are not enough diagrams of anatomical, physiological and diagnostic problems within the central nervous system, which are helpful to the less experienced clinician in this rather complicated field.

GEORGE B. UDVARHELYI

Some Reflections on Genius and Other Essays. By RUSSELL BRAIN. 192 pp., $6.00. J. B. Lippincott Co., Philadelphia, Pa., 1961.

This is an entertaining and informative group of essays on a variety of subjects presenting a wealth of knowledge.

The first speculates on the importance of "schema" or functional organization of the nervous system as a basis for genius. In addition to such neural groupings that deal with conceptual thought the author suggests other characterizing features of genius including hereditary factors controlling verbal symbolization. Why has there never been a woman musical composer of genius?

The second essay deals with interpretations of the medical history of Jonathan Swift. The author suggests "l'Enfant Terrible" suffered from Meniere's disease and in 1742 developed an orbital cellulitis on the left with intracranial extension, possibly a superior petrosal sinus thrombosis, with damage to Brocas area. What Samuel Johnson described as "depravity of intellect" Brain calls an "obsessional personality emotionally arrested at an immature stage."

Then follow seven essays related in one way or another (including an autopsy report) with Samuel Johnson. Though there is no new Johnsoniana presented the author's thorough knowledge of the great lexicographer emphasizes new facets of his friendships and opinions. Of particular interest is the assembling of comments by his contemporaries about him.

One among the remaining essays this reviewer found the most entertaining of them all, titled "Sitting to Epstein". This is a resumé of a probing conversation between Brain and Epstein. Topics ranged from Ruskin, Maynard Keynes, Osbert Sitwell, Wavell, Schweitzer and Beerbohm to Modigliani and Pollock. About each there are observations of interest. Rarely does one find such a scintillating display of breadth of knowledge.

PHILIP F. WAGLEY

BOOKS RECEIVED FOR REVIEW

Allergie und Allergische Erkrankingen. By E. RAJKA. Vol. I, 638 pp., $15.00. Vol. II, 1003 pp., $18.00. Kultura Publications Dept., Budapest, Hungary, 1959.

Annual Review of Pharmacology, Vol. 1, 1961. Edited by W. C. CUTTING. 479 pp., $7.00. Annual Reviews, Inc., Palo Alto, Cal.

Calculations in Pharmacy. By SUE H. ROUSE AND M. GEORGE WEBBER. 234 pp., $5.00. J. B. Lippincott Co., Philadelphia, Pa., 1961.

Ciba Foundation Symposium on Adrenergic Mechanisms. Editors for the Ciba Foundations, G. E. W. WOLSTENHOLME AND MAEVE O'CONNOR. Editor for the British Pharmacological Society, J. R. VANE. 632 pp., $12.50. Little, Brown & Co., Boston, Mass., 1961.

Ciba Foundation Study Group No. 6. Metabolic Effects of Adrenal Hormones. (in honour or Prof. G. W. Thorn). Edited by G. E. W. WOLSTENHOLME AND MAEVE O'CONNER. 109 pp., $2.50. Little, Brown & Co., Boston, Mass., 1961.

District Nursing, 3rd edition. By ELEANOR JEANETTE MERRY AND IRIS DUNDAS IRVEN. 306 pp., $6.00. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents. Bailliere Tindall and Cox, London, 1960.

Haematology. By R. B. THOMPSON. 306 pp., $6.00. J. B. Lippincott Co., Philadelphia, Pa., 1961.

Manual of Clinical Bacteriology. By ALEXANDER KIMLER. 201 pp., $4.75. J. B. Lippincott Co., Philadelphia, Pa., 1961.

Medical Almanac, 1961–62. Compiled by PETER S. NAGAN. 528 pp., $5.00. W. B. Saunders Co., Philadelphia, Pa., 1961.

INTRODUCTORY STATEMENT ON THE OCCASION OF THE DEDICATION CEREMONY FOR THE FINNEY-THAYER WARDS[1]

RUSSELL A. NELSON

Director of The Johns Hopkins Hospital

Dr. TeLinde, Mr. Perkins, Members of The Johns Hopkins Medical and Surgical Association, families of Dr. Finney and Dr. Thayer, and other friends, the Hospital appreciates the opportunity to participate in these meetings and is very proud to be part of the distinction enjoyed by The Johns Hopkins Medical Institutions in medical teaching and research. The Hospital is uniquely related to the clinical aspects of this distinction—both in teaching and in investigation. This morning, we have the pleasure of officially recognizing, honoring, and rededicating our affection for two very distinguished men of our early staff—men who represent, in the highest degree, Hopkins clinical medicine and surgery. We do this by designating a clinical unit in surgery for Dr. John M. T. Finney and one in medicine for Dr. William Sydney Thayer.

In the dedication, we feel it fitting to review and record the work of these men. We have asked two of our doctors to do so—individuals well suited, we believe, to the tasks since they, themselves, represent so much the interests of Dr. Finney and Dr. Thayer. Dr. Harvey Stone will speak of Dr. Finney and Dr. Henry M. Thomas, Jr. of Dr. Thayer.

Mr. Perkins, President of the Hospital and Chairman of its Board of Trustees, will announce the dedication of the units.

[1] February 25, 1961.

JOHN M. T. FINNEY, DISTINGUISHED SURGEON AND EMINENT CITIZEN[1]

HARVEY B. STONE

A few years ago, during an operation in this hospital I asked the instrument nurse for a Bloodgood needle. She said she had never heard of one. Jestingly I asked who Dr. Bloodgood was. "I never heard of him before," was the answer. Yet just a few years before that, Bloodgood was probably known by name, at least, to more people in the United States than any other member of this faculty of medicine. *Sic transit gloria mundi.* Revulsion against time's erosion of the nobility, the great achievements, and the lovable qualities of human beings has led mankind to fight oblivion and to preserve memory and gratitude by a host of efforts. The Egyptian pyramids, the great monuments, the funeral oration of Pericles, and the Gettysburg speech of Lincoln, the memorial windows in the churches, and the simple tombstones in the graveyards, all are ways of saying "Do not forget." Today we mark such an effort by this institution on behalf of two of its famous sons. I have the honor—and I deem it a very great honor—to record in an all too brief allotment of time, some of the reasons why a ward in this hospital is being named the John M. T. Finney Ward. Perhaps there is no one in this audience who has not heard of Dr. Finney, although school and hospital populations change so fast that there may be a number here who never saw him. For them and for all of us, it is a labor of love to outline what Dr. Finney did and to give one man's impression of what he was like. And to submit my qualifications for this task, I may say that I was a student in his classes, an intern on his service, a teacher on his staff, an officer under him in World War I, a junior associate in hospitals, medical societies, social groups, and a personal friend and admirer.

Dr. Finney is known to most people as a great surgeon. Once I was watching him cleaning up an abdomen full of adhesions from multiple previous operations and with several areas of partial or complete obstruction. A famous surgeon standing next to me whispered "Damn Finney. He makes a dreadful job like that look like child's play." He was more than a master technician and a skillful artist with the scalpel. He had to the highest degree the more important qualities of sound judgment, great experience, and solid courage. And from the patient's standpoint the invaluable human characteristics of warmth and sympathy, good cheer and bright humor. Perhaps personal relationship warps this speaker's judg-

[1] Presented on the occasion of the dedication ceremony for the Finney-Thayer Wards, February 25, 1961.

ment, but I think he was the greatest operating general surgeon I have ever known—and I have known most of the outstanding men of my era.

But Dr. Finney was more than a great operating surgeon. He was an interesting and effective teacher, both as didactic instructor and as practical preceptor. For years he carried much of the load of teaching surgery in this institution, and was acting head of the department for a time. Which leads to comment on his interest in education generally. In addition to his personal activity as a faculty member, he served as a trustee to the Gilman School, the McDonogh School and Princeton University. When Woodrow Wilson left the academic for the political world, Dr. Finney was strongly urged by Princeton to become the president of that university. His interest was not limited to the more advanced levels of education as he served for a time as chairman of the school board of Baltimore city.

The latter position was only one of numerous other civic services that he found time to render. For instance, he acted as chairman of the Maryland branch of the American Red Cross, was very helpful in efforts to aid the colored people in their problems, such as securing better hospital facilities. He was called upon for help by many groups, and was often referred to as Baltimore's most valuable citizen. His public services were not limited to this city and state. As a young man, following a local custom long held in esteem, he belonged to one of the Maryland militia regiments. When the gathering shadows of World War I thickened, a number of civilian hospitals were asked to sponsor and organize base hospital units. The Johns Hopkins Hospital accepted such an invitation and set up Base Hospital 18. Dr. Finney was appointed director of this outfit with rank of colonel in the Army Medical Corps Reserve. When we entered the war, this unit was one of the first called into active service, and was actually the first to board an American transport for France early in June 1917. Dr. Finney stayed with the unit as chief of the surgical service until his great qualities were needed in a larger field. He was made chief surgical consultant of the whole American Expeditionary Forces with rank of brigadier-general, and so served for the greater part of the time that American forces in large numbers were in active conflict. Both before and after the war, he was in frequent demand as a consultant to important governmental officials in Washington.

The Church was a great factor in his life. He was not the kind of a minister's son who proverbially turns against his father's teachings. His activity extended from his own church, Brown Memorial Presbyterian, to the nation-wide organization of his own denomination. He served for a time as national vice-moderator of the Presbyterian Church.

If what has been said gives the impression of an ambitious and hyper-

tensive go-getter, it is because only half the picture has been portrayed. Dr. Finney, boy and man, loved games and out-door sports. In college he played varsity football. He played sandlot baseball with the medical students for years. Fishing was a great diversion and rather casual bridge was enjoyed. Instead of being intense he was normally relaxed and easy-going. He loved to hear and to tell good stories and had a notable collection of kindly negro yarns. His use of such resources as comment on current situations was sometimes devastating. A well-known, rather flamboyant surgeon from another city read a paper at a surgical meeting in which he described a very difficult situation that he said he solved "With one bold sweep of my aseptic scalpel." The chairman asked for discussion which was not forthcoming. Then he called on Dr. Finney personally for comment. Dr. Finney rose with evident reluctance. He said that he was not competent to discuss the paper but would tell a story. He told of a negro plowing in a field under a high railroad trestle. A train was crossing the trestle when it went off the track and fell with a dreadful crash to the ground below. The plowman hurried over to the wreck and as he got there a man climbed out of it and began to brush himself off. The darkey said to him, "Mister, ain't you bad hurt?" "Not a bit" said the man. "Well," came this comment, "From now on you best be mighty kereful, case you is done used up all your luck." Dr. Finney simply looked at the reader of the paper and sat down. The audience broke into cheers.

Dr. Finney was a great surgeon, a distinguished citizen, a Christian gentleman, and a fine, mellow human being.

It is most appropriate that this hospital should confer on him the honor of having a ward named after him because he by his life conferred great honor upon this hospital.

WILLIAM SYDNEY THAYER, DISTINGUISHED PHYSICIAN AND TEACHER[1]

HENRY M. THOMAS, JR.

Twenty-seven years ago yesterday, a similar, but much more lengthy ceremony took place when Wards D and E were converted into the new semi-private service and those wards, now being demolished, were named after Dr. Thayer. Judge Harlan, President of the Board of Trustees of the Hospital, stated on that occasion that Dr. Thayer would have been so pleased with this new project since the "charge of five dollars a day" would be the maximum cost to the patient of a bed in these wards. By careful comparison with present day conditions, I note a statistically significant change. On that day, Judge Harlan, President Ames, Dr. Finney and Dr. Simon Flexner left little to be said in portraying the Thayer figure. Here was a Bostonian, bred of long lines of judges, professors, essayists, poets and even brilliant female Greek scholars, who was the soul of honor, loyal among the most loyal, generous, modest, a laboratory scientist and a fine clinician. Now after nearly thirty years it becomes my pleasant duty to recall his great distinction and to recapture something of his rare personality.

The record of honors bestowed on him actually is too long to review at this time. Among his awards were the Distinction Badge of the Red Cross of Russia, the Distinguished Service Medal of the United States Army, and a Commandership in the Legion of Honor of France. He was made fellow or honorary member of more than a dozen foreign medical academies and societies, and was given honorary degrees in Edinburgh, Paris, Montreal, and from several American universities.

Dr. Thayer became well known in America and Europe through his early work on malaria. He had learned from Ehrlich in Berlin how to examine under the microscope preparations of fresh and stained blood and this led to his becoming expert in recognizing and observing malarial parasites. One study, *The Malarial Fevers of Baltimore*, was published in The Johns Hopkins Hospital Reports (1895, vol. 5: 3). He correlated clinical symptoms of tertian, quartan and aestivo-autumnal varieties with the various forms of malarial parasite. He corrected the mortality statistics from the relatively mild form of this disease then prevalent in the eastern states. He also made astute and accurate observations on many clinical entities such as hypertrophic pulmonary osteo-arthropathy and rheumatic heart disease and he, with Francis Peabody and others, was among the first to describe

[1] Presented on the occasion of the dedication ceremory for the Finney-Thayer Wards, February 25, 1961.

and study the 3rd heart sound.[2] He was familiar with all of Laennec's writings and was fond of presenting French books about him to young assistants. In later years, he was consulted not only as a general diagnostician, but more particularly, as a cardiologist. Probably his best known work was that recorded in a monograph entitled *Studies on Bacterial (Infective) Endocarditis* (The Johns Hopkins Hospital Reports, 1926, vol. 22: Part 1, 1) in which he separated 198 cases into their etiological groups and described carefully the various clinical pictures.

It is my hope today to provide a few glimpses of Dr. Thayer which do in fact reveal the essential elements of a great clinician and teacher. A person rarely recognizes himself as the actor that he is. Those constricted by self-consciousness limit their roles to the group pattern and remain safely within the styles of the moment. Not so William Sydney Thayer. He was reared in a family atmosphere of heroic culture, sometimes even of romanticism. Entirely superior to class inhibitions he was superb in portraying the roles he chose for himself. He charmed his listeners whether the mood was professorial, poetic, militant or entirely personal. Doubtless when alone he entertained his audience of one. He did not easily reveal the private core of his emotional substrate. During his last three years he suffered, unknown to all except his personal physician, from attacks of angina pectoris. Knowing his fate, he gave no outward sign.

Dr. Thayer, like many other great teachers, was by instinct an actor; he approached the clinic with a long, swinging stride, coat-tails afloat, carnation in place, a pleased, somewhat detached, smile on his lips, followed closely by the first assistant resident carrying whatever books and paraphernalia he wanted. How we used to enjoy his allowing himself, after reaching the ward, to be completely ensconced in what must have been a kind of surgical gown that covered him from chin to ankle and was tied around the waist, although indeed made of linen that could be starched. His particular Oslerian type of bell stethoscope joined by rather stiff cloth-woven elastic tubes was hooked over the belt of his gown and now he was ready to use both eyes, both ears, both hands and even his sense of smell to complete a meticulous and beautifully executed physical examination. This physical examination did not always stop with the usual methods, but included such procedures as visibly distending the stomach with separately administered portions of effervescing powders. He insisted on accuracy of descriptive phrases for physical signs. Never would he permit the use of the term "hyperresonant" without assuring himself that the user understood that when reduced to exact physical components this meant a percussion note longer, louder and lower pitched than normal, and always during his

[2] *Quelques Remarques sur le troisieme Bruit de Coeur.* Budapest—1909.

teaching exercises there was exhibited a need for the exact use of words. We watched him work and we saw how a cultured intellectual could put an uneducated patient completely at ease, and could help him to give an accurate description of symptoms. We saw how infinite care and thoroughness of expert examination could elicit hidden facts from deep within the body. This was his art. It became our desire to be able to do all of this. Those who were closely associated with him realized that there was no conceit, no snobbishness and that the self-assurance he demonstrated obviously came of long experience and hard work. He brought to the patient precise technique clothed in human understanding and courtly consideration, and his finished production impressed his students.

Dr. Thayer was responsible for the early development of the clinical pathological conferences. With Dr. William MacCallum's enthusiastic cooperation this exercise became the high spot in the teaching program of the medical department. It set the pattern which has been followed so brilliantly ever since.

The importance of medical ethics was not ignored. Surprisingly enough, he served the American Medical Association for ten years or more on its judicial board. He did, in fact, give a lecture to each fourth year class which he called Exigencies of Medical Practice. One such lecture was taken down by Dr. Longcope's secretary, Miss Catherine Hanley, edited by a student and published (The Bulletin of the Institute of the History of Medicine, 1935, vol. 3: 613). Although this does not attempt to cover every facet of medical ethics, it is broad enough in scope to be fascinating reading.

Dr. Thayer adhered to the Oslerian rule which disallowed any critical or demeaning comment about another physician, but this is not to say that he gave silent consent to that of which he disapproved. Indeed at times his displeasure was quite apparent. He advised an optimistic attitude toward any serious disease. He told of several almost unbelievable recoveries from apparently fatal maladies to emphasize the duty of the physician to bring out the hopeful aspects of each case. He took the time to know his patients and, even before it had a name, he was thoroughly aware of psychosomatic medicine.

To realize how extraordinarily effective Dr. Thayer was as clinician and teacher one must recall something of the period of medical practice and more particularly medical education through which he moved. In his time most diseases were poorly understood. Teaching had been didactic, clinical experience was gained after graduation, patients were seen by medical students only at a distance in amphitheaters. For instance, my father, who was Thayer's contemporary, took the prize in his medical school course of obstetrics without ever seeing a baby born. Practicing physicians diagnosed clinical entities such as typho-malaria out of text books and wrote long

prescriptions containing eight or ten ingredients. Thus, the rapid growth in every phase of the study and practice of medicine that young Thayer witnessed must have been so exciting it is little wonder that he changed his allegiance from the study of pathology with Fitz of Harvard and Virchow of Berlin to the new look in clinical medicine and the teaching of Osler. From the moment of his arrival at the Hopkins, Thayer found himself in the forefront of a revivified, dynamic study of medicine. All of his many talents contributed in the development of a great clinician.

One has but to read Dr. Thayer's early articles and even his later lectures given on Pasteur in Paris and on Bright in London to realize that his English was pure and often simple, never obscure and never over-ambitious. Such was his interest in English prose that controversy frequently arose between himself and editors of medical journals to which he submitted professional articles. In fact, when the magazine *Science* refused to revert to his original construction he had little stickers printed which he pasted on each reprint disclaiming responsibility for the English in that paper.

Dr. Thayer's last public address[3] was delivered in 1931 to the graduating class of the new medical department at Duke University on the occasion of planting a sprig of ivy from Sir William Osler's house at 13 Norham Gardens, Oxford, England. He entitled his remarks "The Chief." I shall read to you from this speech since it presents what Thayer himself might have called a glowing picture of the spirit which pervaded the medical clinic of The Johns Hopkins Hospital in those fresh verdant years. After describing Dr. Osler's charm, his ability to cheer people, to stimulate them, to praise the nursing staff, to placate the training school office, to alleviate frustration and yet be able to protect his own valuable time for a large amount of writing and study; after saying all of these things, he goes on to bare the depth of his emotional reaction to The Chief. He says:

"One lived and worked alongside of Osler in an atmosphere of healthy and happy enthusiasm. A week rarely passed without the addition of some bright, amusing remarks, some new epigram or some charming and characteristic incident to the store which we treasured. We loved him and respected him deeply but, treated as companions rather than as students or disciples, we hardly realized how closely he had entered into our lives. I had always expected that he would end his days in the Old Country. I had known how much tempted he had been to accept a call to Edinburgh. I should have fancied that the announcement that he was going to Oxford, while filling me with regret, yet would have come as a piece of news more or less to be expected. When the announcement did come I was hiding in a

[3] A year later he spoke at the opening of the Osler Building.

Washington hotel for several days in order that I might give my time uninterruptedly to some writing. As I read the announcement in the evening paper I was completely overcome. I could think of nothing else, and, despite myself, I lay down on the bed and wept like a child. Others must have had a like experience. I had no idea until that moment, of the part that he had played in my life."

Truly it is fitting that two splendid new wards should be named after these two great men. It is particularly fitting that they should be neighboring wards since Finney and Thayer were life-long intimate friends from the time they started together in the Harvard Medical School. Then they both were house pupils at the Massachusetts General Hospital in 1889, Finney one of four surgical interns and Thayer one of two medical interns in the whole hospital. There were no residents. The following year, while Thayer went abroad, Dr. Finney returned to his native Maryland to start work at the newly opened Johns Hopkins Hospital. When one of Dr. Osler's resident staff resigned Finney recommended Thayer for the post. From then on, despite the differences in their personalities, their careers continued to parallel one another to a remarkable degree. Each, foreseeing our entrance into World War I, had been commissioned in the Army Medical Reserve Corps and, when war was declared, went on active duty with The Johns Hopkins Base Hospital 18. Soon each became the chief consultant to the entire American Expeditionary Forces in surgery and medicine respectively. After the war, each became professor and head of his department. In the daily living of their profession each had the God-given faculty of comforting and cheering people; Dr. Finney often with just a few words of encouragement and a warm friendliness; Dr. Thayer often with an hour of wonderful companionship. I doubt if either could choose a more congenial confrere with whom to go down through the years encouraging new generations of medical people to their highest accomplishment. Hopkins has produced many distinguished doctors and scientists but none who more rightly deserve the honor of exemplifying the Art and Practice of Surgery and Medicine.

CONCLUDING STATEMENT ON THE OCCASION OF THE DEDICATION CEREMONY FOR THE FINNEY-THAYER WARDS[1]

WALTER F. PERKINS

President of The Johns Hopkins Hospital

May I say first that participation in this dedication ceremony today gives me a great deal of personal satisfaction. It was my good fortune to know both Dr. Thayer and Dr. Finney. My acquaintance with Dr. Thayer was quite casual but I knew Dr. Finney much better, having been associated with him in the local Red Cross Chapter. He was a great inspiration to me. His professional competence and executive ability commanded great respect while his warm and friendly personality endeared him to his associates.

Dr. Finney, after graduation from the Harvard Medical School, interned at the Massachusetts General Hospital. He came to The Johns Hopkins Hospital in the spring of 1889 and was a member of the surgical staff when the Hospital opened in May of that year. Dr. Thayer was a contemporary of Dr. Finney at the Harvard Medical School and also at the Massachusetts General Hospital. They were close friends and it was only natural that Dr. Finney would wish their association to continue. He recommended Dr. Thayer to Dr. Osler who invited him to join his staff, which he did in November, 1890. Thus continued what became a life-long association of those two men. As the years went by, these two doctors, one a great surgeon and the other a distinguished physician, served their nation with distinction and brought great credit to The Johns Hopkins Medical Institutions with which they were closely associated during their entire professional careers. It is certainly appropriate that we honor them today.

When it was decided to build the Children's Medical and Surgical Center on the site of the Thayer building—originally one of the public wards—it was necessary to provide substitute semi-private medical and surgical adult beds in another area. This has been accomplished by additions to the Osler and Halsted buildings at a cost of over 1.5 million dollars. It has also increased substantially the total semi-private beds—an objective we have had for a number of years and a promise to our staff which we have carried seriously.

The new Center, in the Hopkins tradition, will be the best that professional and architectural knowledge can create. We believe that there is a great need for superior facilities for the medical care of children and the re-

[1] February 25, 1961.

search activities that will improve such care. We are accepting that challenge. We have in hand nineteen million of the twenty million dollars needed to construct the building and adequately endow this undertaking. We shall ask for bids in April and construction will start immediately thereafter. If all goes well, this 250 bed center will be complete and operating when the Johns Hopkins Medical and Surgical Association meets here again in 1963.

The Board of Trustees was pleased when the Medical Board recommended that the additions to Halsted and Osler be named in memory of two such distinguished former members of our staff, and by appropriate resolution approved the recommendation. So, in the name of the Trustees, I now officially designate the eighth floor of the Halsted building as "THE JOHN M. T. FINNEY WARD" and the eighth floor of the Osler building as "THE WILLIAM SYDNEY THAYER WARD".

SEPTIC CAVERNOUS SINUS THROMBOSIS

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BACKGROUND

Few diseases so depend on their anatomical setting for expression as septic thrombosis of the cavernous sinus. Blood draining from any of the structures of the face, sinuses, mouth, throat, and ears has direct access to the cavernous sinuses through venous channels that contain no valves, and the sinuses themselves are unique in possessing an almost sieve-like framework of connective tissue trabeculations. They are, therefore, susceptible to bacterial seeding in the event of infection in any of the areas mentioned, with subsequent inflammation, and then stasis and thrombosis of the inflowing blood; or alternatively, septic thrombi that have formed superficially may break off and be trapped in the sinus, or may even propagate directly to it.

The underlying anatomy also accounts for all of the major signs of the disease and furnishes the criteria on which diagnosis is based. These are: 1) exophthalmos, chemosis, and eyelid edema, all the result of orbital congestion due to thrombotic blockage of the ophthalmic vein as it enters the cavernous sinus, and probably retrograde infection as well; 2) partial to complete paralysis of the ocular muscles, resulting from involvement of the third, fourth, fifth, and sixth cranial nerves as they pass through the sinus; and 3) the progression of signs from one eye to the other, made possible by virtue of the venous intercommunication between the sinuses.

Since its original description in 1831 (1), only about 300 cases of septic cavernous sinus thrombosis have been reported, and many of these have been questioned by reviewers on the grounds of insufficient diagnostic criteria. It is, of course, likely that many more cases have occurred and have gone unreported for the same reason. Customarily, the disease is categorized into anterior and posterior varieties; anterior, when infection occurs from structures of the face or mouth, and posterior when infection begins in the ears or mastoids. Initiating infections have been reported to occur in all of the theoretically possible sites, although the ears, air sinuses, and nose have been most frequently implicated (2, 3, 4). The majority of infections have been due to the staphylococcus aureus, with occasional staphylococcus albus and streptococcal infections reported, and rarely, even infection from pneumococci or proteus bacilli (3, 4).

[1] Henry Strong Denison Scholar, 1960–61.

Prior to chemotherapy, the usual course was a steady progression of increasingly severe eye signs, rising fever, coma, sepsis, and death within a matter of days or even hours. Although a number of unquestioned recoveries have been acknowledged (3), a review of the literature published in 1936 placed the mortality at close to 100 per cent (2). One of the recoveries was reported in a series of ten cases from the Johns Hopkins Hospital over the period 1889 to 1919 (5). This was a twelve year old boy who on symptomatic therapy alone recovered from the thrombosis as well as a consequent staphylococcal septicemia with metastatic abscesses. A review of the filed histories and autopsies in this hospital from 1919 through 1936 revealed thirteen more cases, none of whom recovered. Six of these cases were reported by Dr. Frank Walsh in an earlier paper (6).

The advent of sulfonamide therapy in 1937 brought an improvement in the prognosis of septic cavernous sinus thrombosis, and by 1943 it had become possible to compile a number of recoveries reported by various authors over the preceding six year period (3). The files of the Johns Hopkins Hospital in this time record ten cases, with one recovery. This was a thirty year old man seen in 1942, who, following several months of headaches, suddenly developed a streptococcal meningitis and bilateral proptosis, chemosis, and ophthalmoplegia. He was treated with sulfadiazine for three weeks, and was discharged with a residual ophthalmoplegia. An underlying pituitary tumor was suspected from x-rays but was never proved, and he was not seen again.

All told, then, in the fifty-four years between 1889 and 1943, a total of twenty-three cases of septic cavernous sinus thrombosis were recorded in this hospital, of whom only two survived. In prognosis, as in regard to infective sites and infecting organisms, these cases conformed to the general experience.

The spectacular change in the outlook for this disease awaited the use of penicillin in the early 1940's. From that time to the present, the number of recoveries has mounted so rapidly that it is currently impossible to find in the literature an unsuccessful case report. Furthermore, the incidence of the disease has become so rare, due most probably to the widespread use of antibiotics in the treatment of superficial infections, that the publication of case series has also disappeared, and given way to single case reports. The coincidence of these two facts opens what appears to be an unqualified achievement to some question, since it is well known that single case reports, in a time when success is possible, tend to be successful. The case series must be coaxed back into style, and the seven cases that follow, representing the experience of the Johns Hopkins Hospital with septic cavernous sinus thrombosis since antibiotics became available, are presented with this in mind. It should be said at the outset that several patients who re-

covered, but who showed an incomplete syndrome, have not been included, as their diagnoses were uncertain.

CASE REPORTS

Case 1. K.L. (#A33242), a three year old white boy, was seen in 1943 following three days of mild fever and anorexia, and three hours of high fever, convulsions, and respiratory distress. On admission he had a fever of 107 degrees, was comatose, and showed a left ear infection, sluggish pupillary reactions, and a slightly dilated right pupil. Blood cultures were drawn and the patient begun on sulfadiazine. During the next twelve hours, bilateral lid edema developed and was shortly followed by a bilateral exophthalmos and chemosis, and the coincident appearance of skin petechiae. Penicillin was then begun at a dosage of 10,000 units every three hours. Over the next three days, eye movements became limited and finally paralyzed, a ptosis of the right eye appeared, and the fundi showed venous engorgement and papilledema. Under continuing penicillin therapy, however, the fever gradually subsided, and the eye signs regressed to a mild right eye ptosis and limitation of ocular movement, which remained at his discharge three weeks after admission. Blood cultures grew out hemolytic staphylococcus aureus; spinal fluid was normal throughout his hospital stay.

Over the years, the patient has continued to show a mild right ptosis, as well as a pseudo-Graefe sign (elevation of the upper lid when the eye is adducted). This is a particularly unusual consequence of inflammatory involvement of the third nerve and indicates a misdirected regeneration (9). Otherwise, he is entirely normal.

Case 2. M.C. (#315479), was a forty-two year old white man who had had several episodes of aching and partial deafness in his left ear in the weeks prior to his admission in 1944. His last episode was attended by a headache, and then, in rapid succession, a stiff neck, shaking chill, and delirium. When seen, he was semicomatose, with rapid respirations and pulse, and a temperature of 104 degrees. He made purposeless movements and responded only to pain. A fluctuant swelling over the left mastoid was noted, as well as a left otitis media. Examination of the eyes was normal. Lumbar puncture demonstrated a pneumococcal meningitis, and a myringotomy produced pus that also grew out pneumococci; in consequence of this he was begun on intravenous penicillin, 600,000 units, and sulfadiazine. Four hours after admission, however, his left eye was observed to have become proptosed and chemotic. A left mastoidectomy was performed, following which the proptosis increased and the eye became markedly edematous. Twenty-four hours after admission, he began having generalized convulsions, his breathing became very shallow, and he died.

Autopsy. An acute purulent left otitis and mastoiditis, and a generalized purulent meningitis were found, from all of which pneumococci were cultured. Both the left cavernous and left lateral sinuses contained purulent thromboses. The brain and hypophysis were normal. The left eye showed some papilledema and contained an intravitreous hemorrhage. There was cardiac enlargement and dilatation, and a lobular pneumonia.

Case 3. B.Y. (#339299), was a forty-two year old colored woman who had had headaches for several days prior to the abrupt appearance of an acute pansinusitis on the right, with an associated right third nerve palsy, and meningismus. Following a frontal trephine and ethmoidectomy, her condition worsened, and she was transferred to the medical service with a temperature of 102 degrees, and signs of meningitis. At this time she showed marked bilateral edema of the eyelids, some left eye proptosis, and a com-

plete left third nerve palsy. The third nerve on the right side, which had been involved initially, was normal. Lumbar puncture produced a cloudy fluid under high pressure, with many polymorphonuclear cells, but was sterile. Twenty-thousand units of penicillin were instilled intrathecally, and 20,000 units were begun intramuscularly every three hours, in addition to sulfadiazine, but her fever and headache continued unabated for two days, at which time the extraocular movements of her left eye diminished, and she began losing the hearing in her left ear. The feeling was that thrombosis was proceeding from the left cavernous sinus to the left lateral sinus, and it was decided to open the sphenoidal sinus to allow drainage. This was performed twice, each time producing pus from which alpha and beta streptococci and staphylococcus albus were cultured. The large amount of pus obtained on the second aspiration was felt to indicate a communication between sphenoid and cavernous sinuses, and drainage tube was left in place. The patient's temperature became normal the next day, and was followed by a more gradual disappearance of all her other signs and symptoms in an ensuing two week period.

Case 4. E.E. (#448226), was a thirty-one year old colored woman, who one month prior to her admission in 1948 had an upper respiratory infection. Nasal congestion persisted and was in subsequent weeks complicated by frontal headaches and pain in the left eye. On the morning of admission she awoke with a painful swelling of the right eye and experienced chills and fever. When seen she was acutely ill, with a fever of 104 degrees, and the right side of her face was hot and swollen. The right nasal cavity was occluded and purulent. The right eye was proptosed, with eyelid edema, chemosis, complete ophthalmoplegia, and no light perception; the fundus showed some papilledema and retinal blanching. The left eye was normal. The spinal fluid, although it contained some polymorphonuclear cells, was sterile, as was the blood. Sulfamerazine and penicillin (1.6 million units daily) were begun, but after four days she was no better and in fact had developed a sixth nerve palsy of the previously normal left eye. Because x-rays showed bilateral ethmoid clouding, an ethmoidectomy was then performed, and streptomycin was added to her therapy upon learning that cultures of both the nasopharynx and right conjunctival sac had grown out Hemophilus influenza. Over the next two weeks her fever subsided, an abscess in the right orbit localized and was drained, and all signs gradually disappeared.

Three weeks after her discharge, however, some friends found her at home ill, vomiting, and with a stiff neck. As she shortly became unresponsive, they brought her to the hospital, where she was seen to be completely comatose, with a 106 degree fever, rapid pulse, and Cheyne-Stokes breathing. She had signs of meningitis, and a right hemiparesis. Her right eye was again proptosed and chemotic, and her left eye showed a partial third nerve palsy. She was quickly begun on penicillin and streptomycin, but steadily deteriorated and died fourteen hours after admission.

Autopsy. The positive findings were confined to the head. Abscesses were found in the right orbit and in the left frontal lobe, and the left cavernous sinus was filled with a fresh purulent thrombus. The right cavernous sinus and hypophysis appeared normal but were not studied microscopically. There was a widespread purulent meningitis. Both the spinal fluid and frontal lobe abscess grew out staphylococcus albus, and in addition the frontal lobe abscess grew a pleomorphic gram-negative rod, thought but not proved to be Hemophilus.

Case 5. O.H. (#631842), a sixty-four old white woman, was seen in 1952 with a long history of sinusitis and sinus operations, and transferred to the medical service shortly after an ethmoid operation. She was lethargic and had a temperature of 104 degrees, showing a left-sided conjunctival and periorbital edema, a complete left ophthalmo-

plegia, and meningitis. Spinal fluid grew out pneumococci and the patient was given 20,000 units of penicillin intrathecally and begun on 24 million units of penicillin and two grams of streptomycin daily with good response so that by the fourth hospital day she had become mentally clear, and by the end of the first week the eye signs, which had become bilateral, had largely disappeared. Her subsequent course was complicated only by a persistent low-grade fever associated with an ulcerative stomatitis that ultimately cleared under appropriate antibiotic therapy.

Case 6. E.L. (#733583), was a forty-seven year old white woman seen in 1956 with a three day history of progressive swelling and redness of her nose that was followed by some orbital swelling, fever, chills, and vomiting. She presented with a 105 degree temperature, was drowsy, and had bilateral periorbital and conjunctival edema and redness, left eye proptosis, and a left sixth nerve palsy. Draining pustules were noted inside the nose. She was immediately given 20,000 units of penicillin intrathecally, and 40 million units of penicillin with one gram of streptomycin was begun intravenously. The following day, cultures of the nasal pus, blood, and CSF, which had been obtained on admission, grew out a hemolytic staphylococcus aureus that was found to be mildly sensitive to chloramphenicol, Bacitracin®, and Neomycin®, moderately sensitive to Erythromycin®, and wholly resistant to all other drugs tested. With the addition of Erythromycin®, her fever slowly came down, and her sensorium gradually cleared; by the tenth hospital day her eye signs had almost entirely abated. Her subsequent course was marked by the development of a left pleuritic pain and friction rub, and a palpable spleen, felt to be the result of septic emboli, but they both cleared and the patient was discharged after a four week stay with only a mild ptosis and residual paralysis of the left lateral rectus muscle.

Case 7. P.S. (#970772), seen in 1960, was a forty year old white woman who was brought to the accident room with a three day story of having picked at a sore in her nose and thereafter experiencing headaches, feverishness, nausea and vomiting, diplopia, and finally delirium. When seen, she was completely comatose and in a decerebrate posture, showed signs of meningitis, and had a fever greater than 107 degrees. She showed striking bilateral orbital and facial edema, proptosis, conjunctival swelling and hemorrhage, and complete ophthalmoplegia. Under vigorous hypothermia, her temperature was brought down to 93 degrees, and she was quickly begun on 40 million units of penicillin and four grams of chloramphenicol in a rapid intravenous drip. Six hours after admission, however, she began having intermittent grand mal seizures; three hours later her blood pressure had to be maintained with vasopressors. She continued to deteriorate and died twenty hours after admission. Some time later the original cultures of the nares, blood, and CSF were reported as growing out a hemolytic staphylococcus aureus that was completely resistant to penicillin and only slightly sensitive to chloramphenicol; it was, however, very sensitive to Erythromycin®, Vancomycin®, and Ristocetin®, and extremely sensitive to Staphcillin®.

DISCUSSION

These seven cases were collected by reviewing all of the medical histories and autopsies from 1943 through 1960 that were catalogued as intracranial venous sinus thromboses. A total of 425,000 hospital admissions occurred during this period. For autopsied cases, the criteria for sepsis were simply the presence of pus in the thrombosed sinus, or a positive culture from it;

in those cases not coming to autopsy, the criteria were those eye signs mentioned in the opening remarks of this paper. Although proven septic cavernous sinus thrombosis can occur unilaterally, attested by the autopsy on Case 2, the alternative diagnosis of orbital cellulitis cannot be excluded in patients for whom only clinical evidence exists, and such cases have not been included in this series. This restriction, together with the possibility that mild degrees of thrombosis may occur without any eye signs at all, recover, and so never be suspected, means that the actual number of septic cavernous sinus thromboses must certainly be larger than can be proved.

It appears from an analysis of the seven cases presented that neither the origins nor bacterial etiology of the disease have changed much from what they were in years past. Three of the cases originated from sinus infections, two from ear infections, and two from nasal furuncles. Staphylococcus aureus was implicated three times, pneumococcus twice, and staphylococcus albus twice, one of these in conjunction with a streptococcus, and the other most probably with Hemophilus influenza. Both patients with nasal furuncles had infections with staphylococcus aureus, but there is no clear association of a given organism with one or another site of infection; nor does there appear to be any sharp distinction that can be drawn between those patients who died and those who survived. Perhaps the most that can be said is that the former were, even on presentation, the more desperately ill; quite possibly they were beyond help.

The last case may nevertheless be used to illustrate a difficult problem, for sensitivity reports were not in time to be of use in guiding her therapy, and in the absence of appropriate chemotherapy, the prognosis for this disease reverts to what it was thirty years ago. Yet today, because of bacterial resistance to many drugs, the probability of predicting the appropriate antibiotic in advance of bacteriological reports is diminishing. The answer is not, as has been suggested as recently as 1959, to wait for the reports before initiating therapy (10). An educated guess is required, and the drugs selected must be given immediately in this as in any other disease with a potentially fulminant course.

Realizing the probability that the offending organism will be a gram-positive species, the choice has in recent years been penicillin, with chloramphenicol and streptomycin included as adjuncts in case of other organisms or penicillin resistance. It now appears that resistance to chloramphenicol is sufficiently prevalent to warrant treating this disease at the very outset with additional drugs, effective against gram-positive (and particularly staphylococcic) organisms, that are customarily withheld until resistance to the first-line drugs has been demonstrated. A suggested approach is the combination of penicillin G and Staphcillin®. The peni-

cillin should be effective against the streptococcus and pneumococcus, while the Staphcillin® could be expected to be effective in the treatment of a resistant staphylococcal infection.

One other possibility for therapeutic assistance lies in the use of steroids. In a review of six carefully autopsied cases occurring in the 1930's, Dr. Walsh emphasized the frequent involvement of the hypophysis in the inflammatory process of septic cavernous sinus thrombosis (6). The two autopsied cases presented here were said not to show any hypophyseal involvement, but serial sections were not made, and so it cannot be said with certainty that no inflammation was present; it is, of course, also possible that functional derangements may occur with minimal or absent organic changes. The opportunity for adrenal insufficiency on this basis, contributing to the kind of vascular collapse experienced by the last patient, is a distinct possibility. In future cases it would be interesting to obtain eosinophil counts and steroid assays during the course of the disease in order to clarify this problem; in any event, the use of steroids as an adjunct in supportive, and possibly specific, therapy deserves notice.

The prognosis, judging from this series, must still remain guarded.

SUMMARY

The close anatomical dependency of both the occurrence and manifestations of septic cavernous sinus thrombosis is indicated, and the spectrum of initiating infections and causative organisms is reviewed. The pre-chemotherapeutic experience of the Johns Hopkins Hospital is described briefly, and is followed by case reports of seven patients seen with septic cavernous sinus thrombosis since antibiotics have become available. The current trend of drug resistant organisms, in conjunction with a fulminating course in the absence of appropriate antibiotic therapy is emphasized, and a plea made for the immediate use in such patients of additional drugs usually held in reserve until sensitivity reports have returned. Steroid therapy is mentioned as another possibly helpful measure. The prognosis, although much improved by antibiotics, is still not so optimistic as recent case reports would lead one to believe.

ACKNOWLEDGMENT

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WHIPPLE'S DISEASE: A NOTE REGARDING PAS-POSITIVE GRANULES IN THE ORIGINAL CASE

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It is now accepted by most authors that cells containing periodic acid-Schiff (PAS)-positive granules are found in all bona fide examples of Whipple's disease and that these cells help distinguish it from other diseases of malabsorption (1, 2, 3, 4). In addition to its importance for diagnostic purposes, the demonstration of cells containing the PAS-positive substance in a variety of tissues in patients with Whipple's disease has led to a re-oriented concept of the disease. The PAS-positive substance, which is usually considered to be an as yet poorly defined glycoprotein, may have a fundamental bearing on the pathogenesis of the disorder. Emphasis has correspondingly shifted away from the striking lipid accumulations in the intestine and lymph nodes which led to Whipple's designation of the disorder as *lipodystrophy intestinalis* (5).

However, it has never been known with certainty that the first case reported contained cells having the PAS-positive granules, for the PAS staining technique, being a relatively recent development, was not available to Whipple. Therefore, tissues from the original case described by Whipple from The Johns Hopkins Hospital in 1907 (5) have been restudied using the PAS reaction.

Paraffin embedded and alcohol preserved tissues from the original case (Autopsy No. 2883) were available. Sections of intestine, lymph node, heart and pancreas were stained with hematoxylin and eosin and by the periodic acid-Schiff methods (6). Intestine and lymph node from the case described by Jarcho (7) (Autopsy No. 13858), on which the PAS stain had never been performed, and tissues from a recently diagnosed case in a forty-seven year old man (Surgical Path. No. 59-1746, Autopsy No. 30013) served as control material. Both control cases contain cells showing typical PAS-positive intracellular granules. Small intestine and stomach from the biopsied case were also used to perform a Levaditi silver stain (6).

The principal artefact evident in sections of small intestine from Whipple's case after fifty-four years is marked distortion and loss of mucosal cells; other cells are in a good state of preservation though the HE

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staining is somewhat weak. Typical "club-shaped" villi are apparent; these show the large vacuoles previously occupied by fat and also many clumps of the pink "foamy" cells (Fig. 1) described by Whipple. With the PAS stain these "foamy" cells are found to be filled with brilliant red-violet granules (Fig. 2) precisely like those in the control material. A few such granules are scattered in the intercellular space. Lymph node tissue from Whipple's patient similarly still reveals "foamy" cells that show the PAS-positive material (Figs. 3 and 4). Such cells are frequently grouped near large fat vacuoles, but are noted in all parts of the stroma, including the germinal centers.

Occasional cells that often resemble fibroblasts or histiocytes and contain PAS-positive granules are also seen in the myocardium and epicardium (the site of a chronic adhesive pericarditis). Similar cells are observed in the pancreatic interstitial tissue. These findings are in agreement with recent reports describing such cells in many organs from patients with Whipple's disease (3, 4).

The original description by Whipple of the disease that bears his name stands as a classic of clarity, objectivity and completeness. Many avenues of investigation as to the nature of the disorder were explored by him, and if the PAS stain had been available at that time, it seems reasonable to suppose that he would have described intracellular granules positive for that reaction. For Whipple clearly stated that the osmic-positive lipid droplets and globules are primarily extracellular and within the lymphatic channels and noted the contents of the pink "foamy" cells for the most part to be non-lipid by ordinary staining criteria. This feature of the original case was not properly appreciated by some authors but has been perceived by Black-Schaffer (1) and those investigators writing about Whipple's disease more recently.

In the course of his study of the original case, Whipple stained formalin-fixed lymph nodes by the Levaditi silver technique, a stain ordinarily used to demonstrate spirochaetal organisms. As with most stains based on reduction of silver salts to metallic silver, its specificity is only relative. By using this stain, Whipple found many small extracellular and intracellular silver-staining particles; he noted that the intracellular particles were particularly evident within the "foamy" cells while many of the extracellular granules were rod-shaped and accumulated in the fat vacuoles. Without concluding that the argyrophilic particles necessarily represented an organism, Whipple stated, "Whether this is the active agent in this peculiar pathological complex cannot be determined from the study of this single case, but its distribution in the glands is very suggestive".

Whipple's original Levaditi-stained slides are not available, but a

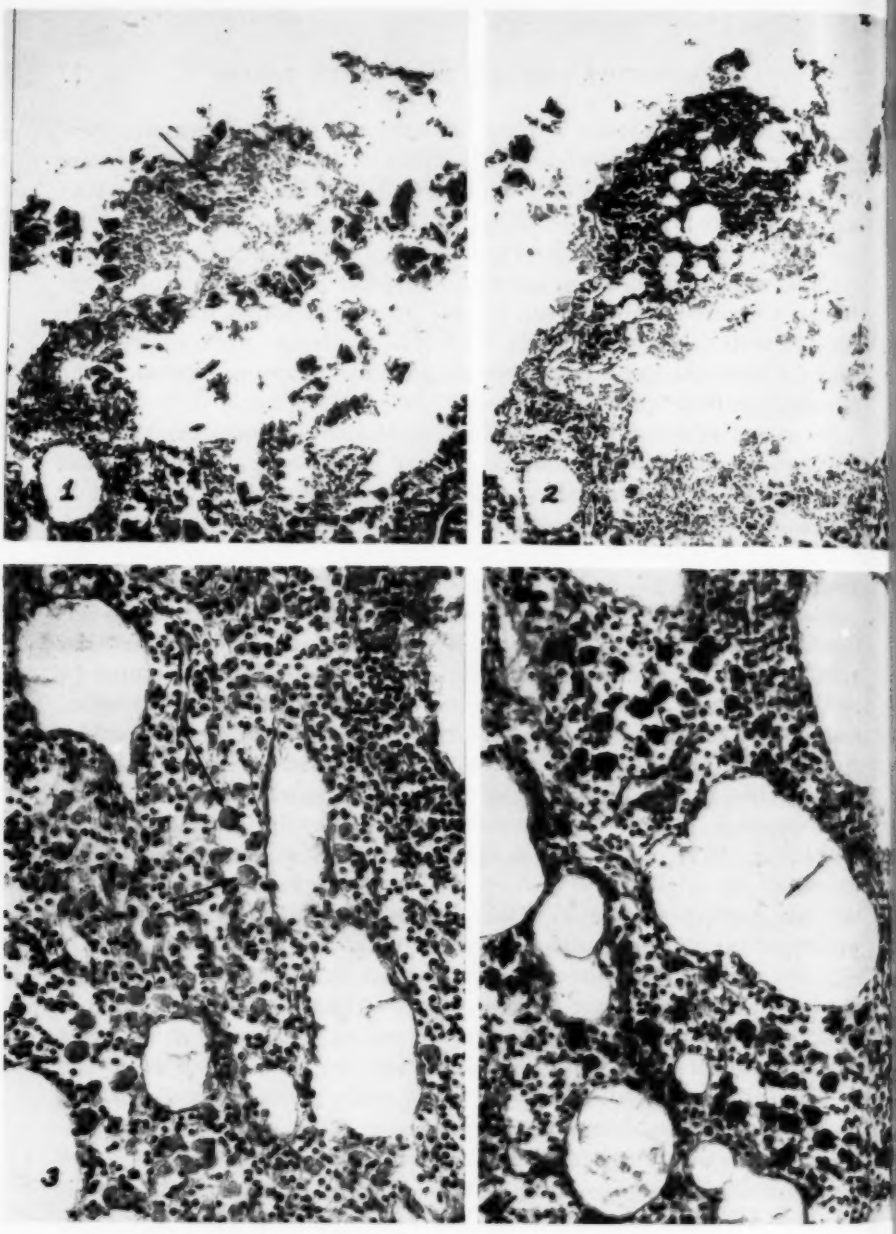

FIG. 1. A clubbed intestinal villus from the original case of Whipple's disease showing fat vacuoles and replacement of the lamina propria by "foamy" cells (arrow). Hematoxylin and eosin, 100 ×.

FIG. 2. PAS stain of villus seen in Figure 1. The "foamy" cells are strongly PAS positive. 100 ×.

FIG. 3. Lymph node from the original case demonstrating large, rounded "foamy" cells like those in the intestine (arrows). Hematoxylin and eosin, 250 ×.

FIG. 4. A comparable area of the lymph node seen in Figure 3 showing "foamy" cells crammed with PAS-positive granules. Periodic acid-Schiff stain. 250 ×.

Levaditi stain performed on intestinal and stomach tissue from our most recent case (Autopsy No. 30013) demonstrates the presence of small intracellular and extracellular argyrophilic particles that correspond closely to those depicted by Whipple. The possible relationship of this generally overlooked observation of Whipple's to the PAS-positive granules and to current findings using electron microscopy, is indicated in another report from this laboratory (8).

Confirmation of the presence of cells containing the PAS-positive granules in the original case of Whipple's disease further unifies such cases and adds weight to the pathognomonic significance of the peculiar PAS-positive granule-containing cells.

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COMBINED ELECTRON AND LIGHT MICROSCOPY IN WHIPPLE'S DISEASE[1]

Demonstration of "Bacillary Bodies" in the Intestine

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In 1949, Black-Schaffer (1) first called attention to an intracellular PAS-positive substance, presumed by him to be a glycoprotein, in the intestine and lymph nodes of patients with Whipple's disease. This finding has since directed attention away from consideration of lipid metabolism in Whipple's *lipodystrophy intestinalis* (2) and focused attention on his "foamy cells"; these he originally recognized as being mainly non-lipid containing. Subsequent studies have confirmed the observation that the intracellular PAS-positive material is most heavily concentrated in the intestines and mesenteric lymphatic system and have pointed out that cells containing PAS-positive granules may be found in virtually every organ in the body (3).

Whipple's disease, therefore, is considered a systemic disease by pathologists and clinicians, and it is believed that the PAS-positive substance is of major significance in its pathogenesis.

In spite of this approach, the etiology of Whipple's disease as well as the origin of the granular PAS-positive substance is still a mystery. The many points of similarity between Whipple's disease and the "storage diseases" have led to widespread belief that it may be a disorder of this type (3, 4, 5). This interpretation was also suggested by the authors of one electron microscopic study of Whipple's disease (4). On the other hand another report (6) gives a different interpretive emphasis to the ultrastructural features of Whipple's disease: certain extracellular bodies that may be micro-organisms.

In the present paper ultrastructure observations on peroral jejunal biopsies from a patient with Whipple's disease will be described and com-

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pared with light microscopic observations. Our findings suggest that Whipple's disease may either be a peculiar infection due to a microorganism most closely resembling a bacterium, or, even if the organisms are not the *primary* causative factor, that they may play a role in genesis of the PAS-positive granules.

MATERIAL AND METHODS

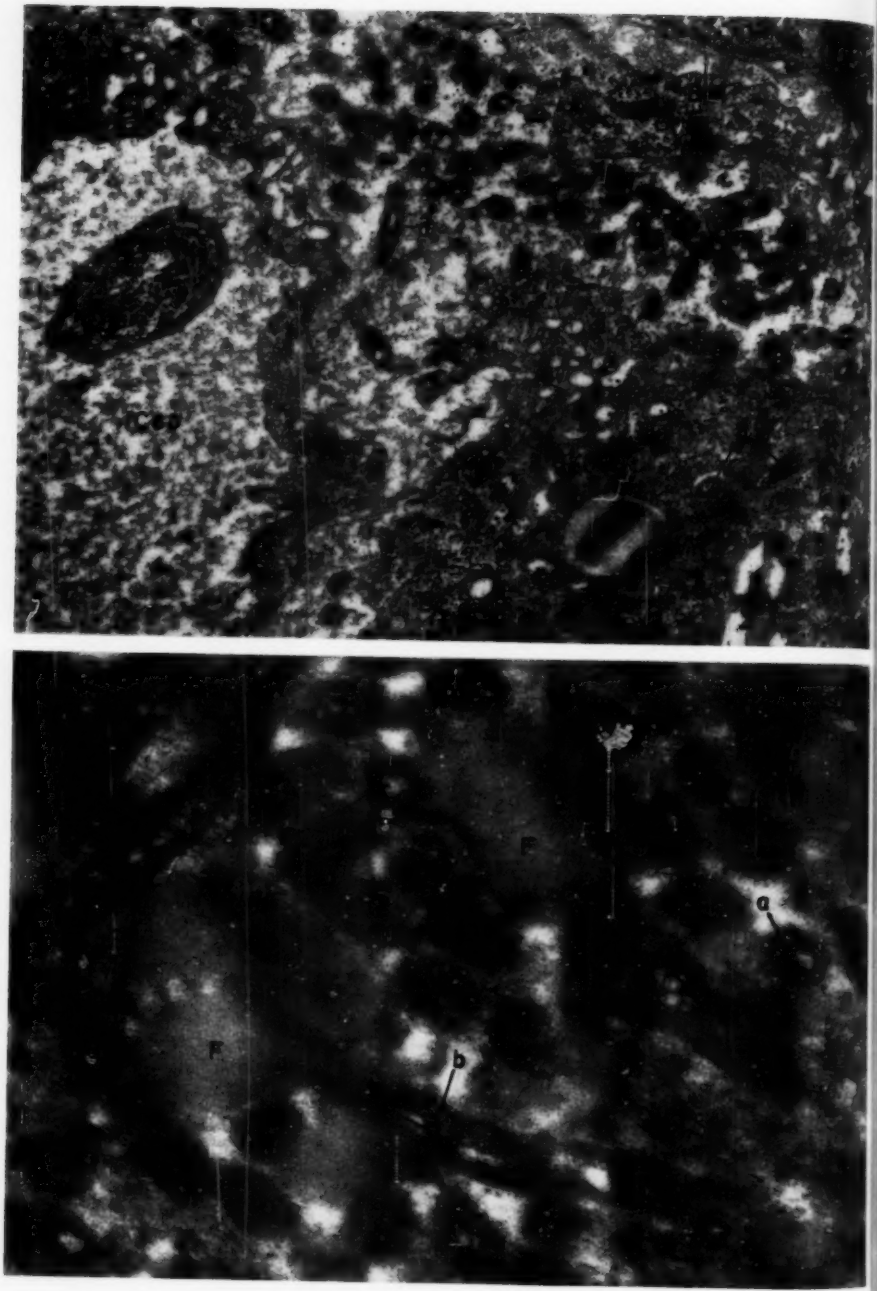
Source of Tissue: The electron microscopy studies and most of the light microscopy studies were performed on jejunal tissue obtained by peroral Crosby capsule biopsy (7) from a patient (JHH #840653) whose jejunum showed many PAS-positive granule containing cells on multiple biopsies. The patient was a forty-seven year old farmer who had episodes of diarrhea, weight loss and migratory arthralgias for five years. During four hospital admissions he passed anywhere from one to fourteen stools per day but always had steatorrhea. Small bowel X-ray series demonstrated the malabsorption pattern. Routine laboratory studies were normal except for anemia. The serum carotene and d-xylose excretion were low (9.0 gamma per cent and 3.1 gm. per five hours respectively). Fecal fat excretion ranged from 6.6 to 36.5 grams per day. During the last one and one-half years of his illness, this patient's weight fluctuated from ninety-eight to 136 pounds. He died during an exacerbation of his illness.

An autopsy (JHH #30013) confirmed the diagnosis of Whipple's disease, and some of these tissues were also used in the present investigations.

Electron Microscopy: Portions of fresh jejunal tissue were cut into approximately 1 mm. cubes and fixed immediately in 1 per cent pH 7.4, isotonic osmic acid solution at 0°C. The fixation period was twenty-five minutes. Dehydration was performed in graded ethyl alcohol solutions beginning with a 70 per cent concentration, and embedding was done in a prepolymerized mixture of 10 per cent methyl and 90 per cent butyl methacrylate. Blocks were trimmed first in a rough manner and used to make 1 micron sections with a glass knife on the Porter-Blum microtome. These "thick" sections were mounted on slides and stained for the PAS reaction (see below). The block was then retrimmed to a small truncated pyramid while using the previously stained "thick" section as a guide to a suitable region of each block. Ultrathin sections were cut with a diamond or glass knife on the Porter-Blum microtome and at least one grid was routinely stained with uranyl acetate (8). Some sections were also left unstained or stained with lead hydroxide (9). The grids were examined in an RCA EMU-2A electron microscope equipped with an intermediate lens, centerable objective and condenser apertures and an electrostatic compensator.

Light Microscopy: In addition to the ultrathin sections from each trimmed block of intestine, a final "thick" section (about 1 micron) was made for light microscopy. The "thick" sections, with appropriate staining, were used for correlative light microscopy studies of the same areas seen in the electron microscope.

All staining on osmic-fixed and methacrylate-embedded "thick" sections was done after removing methacrylate by either one hour treatment with chloroform at 56°C. or tetrahydrofuran for fifteen minutes at room temperature. The PAS staining reaction was very successful, requiring only that each step in the technique (10) be prolonged by a factor of three or four. Hematoxylin was used as a counterstain. Various silver staining techniques were attempted on the osmic fixed tissue but without great success.



FIGS. 1 AND 2

The periodic acid-methenamine silver (11) and Gomori reticulin stains (10) were performed on either ordinary paraffin sections of formalin fixed jejunal biopsy material or on 1 micron sections of similar tissue that had been embedded in methacrylate after paraffin extraction.

Also studied were formalin or Zenker-formalin fixed, paraffin embedded sections obtained by biopsy or at autopsy and stained for bacteria by the Brown and Brenn method, the Kinyoun's acid-fast stain, Giemsa stain, Warthin-Starry and Levaditi spirochete stains, and Machiavello's rickettsial stain (10).

RESULTS

Electron Microscopy

Extracellular Bacillary Bodies

Electron microscopy demonstrates many curious rod-shaped bodies in the extracellular space surrounding cells of the lamina propria from our case of Whipple's disease. It will be shown that these bodies have many of the morphologic characteristics of bacteria and hence they will be termed "bacillary bodies".

The "bacillary bodies" are noted at all levels of the lamina propria, but often are concentrated in tightly packed swarms just beneath the epithelial basement membrane (Fig. 1). They also may appear in large numbers around many of the PAS-positive granule containing cells. Osmiophilic fat droplets are numerous within the extracellular region too, but aside from occasionally obscuring the "bacillary bodies", no special relationship between them is observed. Many of the "bacillary bodies" appear round or slightly ellipsoidal in the electron micrographs; hence they do not exclude a cocco-bacillary form. However this appearance in the electron micrographs is no doubt largely if not entirely explained by tangential and cross-section cutting of the bodies since it is apparent that the extreme thinness of electron microscopy sections (50 to 100 millimicra) sharply limits full length visualization of *any* randomly arranged, long, rod-shaped structures. Findings in the light microscope (see below) tend to support the conclusion that certainly many if not all of the bodies have a bacillary form.

FIG. 1. Jejunal lamina propria in Whipple's disease. Extracellular (a) and intracellular (b) "bacillary bodies" are seen along with an adjacent capillary (Cap). The normal epithelial basement membrane (BM) is seen at the top of the photograph. Uranyl acetate stain. 19,000 ×.

FIG. 2. Details of extracellular "bacillary bodies". One "bacillary body" (a) clearly shows an outer jacket, two limiting membranes and inner granular substance with lighter areas and a tendency to vacuole formation; another (b) demonstrates the great length that may be seen (about 1 micron). A prominent vacuole (V) is seen in still another "bacillary body", and there are extracellular fat droplets (F). Uranyl acetate stain. 56,000 ×.

The greatest "bacillary body" length measured in the electron micrographs has been slightly more than 1 micron. Their width is extremely uniform, measuring 0.15 to 0.20 micron.

Well-preserved tissue from the present case of Whipple's disease shows the "bacillary bodies" to have uniform structural features (Fig. 2). They have a moderately dense outer jacket about twenty-five millimicra thick. This outer jacket has so far shown only an indefinite peripheral demarcation, but it is limited on its inner side by a dark, thin membrane which completely encompasses the contents of the "bacillary body". Adjacent to this is a clear zone measuring 6 to 7 millimicra that lies between the outer membrane and a less distinct inner membrane. The interior consists mainly of very electron-dense material which has a rather ill-defined, coarsely granular appearance, but centrally the "bacillary body" reveals one or more indefinite areas of lesser density and granularity. These frequently are seen as large, often sharply outlined, and completely clear central vacuoles. The latter condition could result either from degeneration or an artefact of poor preservation.

PAS-positive Granules

Great interest, of course, centers around the peculiar cells containing PAS-positive granules, generally considered histiocytes, that are found in abundance in the intestinal lamina propria of patients with Whipple's disease. The ultrastructure observations to be described below indicate that the PAS-positive granules in our case assume a variety of forms which can be conveniently described as an orderly sequence or spectrum of characteristics. Whether or not such a sequence has any meaning in terms of actual granule transformation remains conjectural.

The intracellular granules, which are PAS-positive by light microscopy, vary greatly in size, the smallest measuring somewhat less than one micron in diameter and the largest at least six micra. Considerable variation in granule size may be found within a single cell, or from one cell to the next.

The granule-containing cells often show isolated "bacillary bodies" lying free within their cytoplasm (Fig. 1). It is also readily evident from study of random micrographs that a few intracellular granules containing "bacillary bodies" are regularly present. Such granules appear in the electron micrographs as intracytoplasmic structures that are limited by a thin outer membrane and contain several of the "bacillary bodies"; the intragranule "bacillary bodies" may show the usual outer jacket but even more often only the membranes and central granular material. "Bacillary bodies" frequently look enlarged or swollen when present in the intra-

cellular granules, and may have a large central vacuole (Figs. 3 and 5). The "bacillary bodies" in many granules are embedded in a moderately dense amorphous matrix, particularly when the "bacillary bodies" are readily recognizable as such.

In a general way as the "bacillary bodies" become increasingly difficult to recognize (those present often lacking an outer jacket or showing other loss of detail), the moderately dense amorphous matrix around the "bacillary bodies" is less evident. A curious network of delicate, thin lines is then increasingly apparent.

Finally there are many granules, that show no recognizable "bacillary bodies" and only the delicate network of lines placed against a very non-electron dense background. This is the commonest type of granule (Figs. 6 and 7).

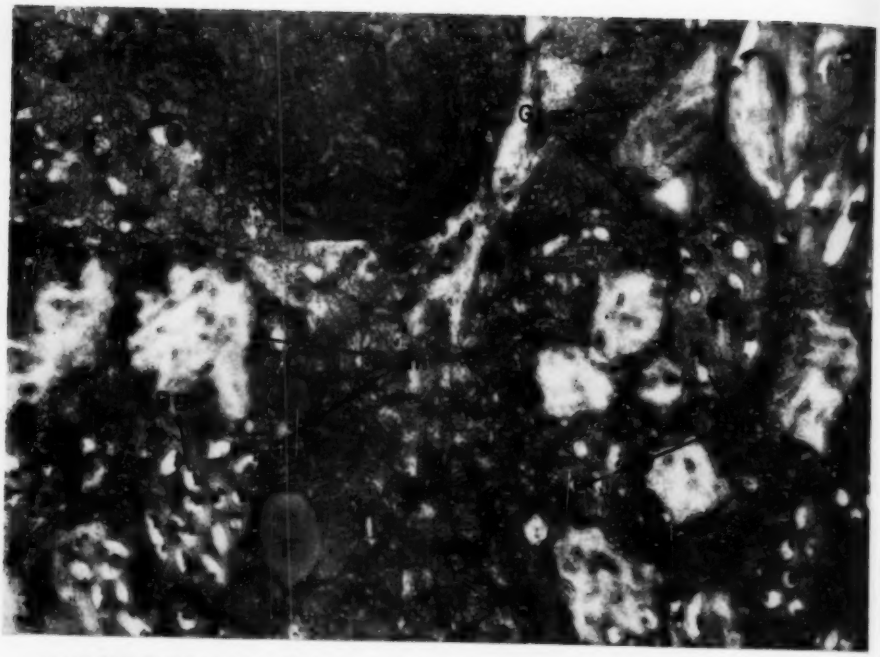
It cannot be determined whether the thin lines composing the network are thread-like or membranous, but most electron microscopists would probably look upon them as membranes. They curve in all directions through the granules sometimes forming small circular or ellipsoidal loops or occurring as parallel, undulating pairs. The lines or membranes usually show a spacing of approximately 20 millimicra. The exact relationship between the network of lines and any enclosed "bacillary bodies" is not too clear from the electron micrographs, but at times the lines seem to merge imperceptibly with the "bacillary bodies". It is even conceivable that they are derived from membranes in the "bacillary bodies".

The Granule Containing Cell

The cells containing PAS-positive granules also show multiple large mitochondria and many small, dense cytoplasmic granules which are in part formed into small clusters or are lined up along membranes to comprise an endoplasmic reticulum (Fig. 5). A few homogenous osmiophilic fat droplets may also be noted within the granule-containing cells. However, none of these other intracytoplasmic structures has shown regular spacial relationship to the large PAS-positive granules. Also, the "bacillary bodies" are distinctly different from any recognizable intracellular organelle. No definite evidence of either phagocytosis or extrusion of the granules or "bacillary bodies" by the cells has been noted in this study.

Epithelial Cells

Occasional clusters of "bacillary bodies" may be seen inside the epithelial cells, yet no typical PAS-positive granules with network forma-



FIGS. 3 AND 4

tion have been found there (Figs. 4a and 4b). The outer cell membranes, intracytoplasmic structures, secretion vacuoles and microvilli composing the brush border of the epithelial cells all appear normal as does the epithelial basement membrane.

Light Microscopy

PAS Staining Reaction

PAS staining of 1 micron sections made from osmic acid fixed and methacrylate embedded tissue is a useful tool in the study of Whipple's disease. The very thin sections with their excellent preservation provide striking delineation of detail (Figs. 8 and 9). A notable feature of the granules in such a preparation is their variation in staining intensity. This variation is to some extent proportional to granule size, the deepest coloration often being found with the largest granules. However, even the smallest granules are stained, and all have a more or less homogenous appearance.

As indicated by the electron microscopy, "bacillary bodies" have dimensions that approach the resolution of light microscopy. This fact, coupled with their tendency to occur as tightly packed swarms, renders demonstration of isolated "bacillary bodies" difficult. But large extracellular *clusters of* "bacillary bodies" are easily recognized when present, and their identification as such has been repeatedly corroborated in the present case by correlative electron microscopy of the same field in adjacent sections. PAS staining of "bacillary bodies" is moderately intense and similar to that of the smaller intracellular granules, yet often less than that of the large-sized granules. One of the best demonstrations of isolated "bacillary bodies" in a PAS stain has been achieved in an ordinary Zenker-formalin fixed paraffin section (Fig. 10). Their small size and rod-shape are evident.

Specific Stains for Organisms

In two separate jejunal biopsies from the present patient done several months apart, bacterial stains have shown large, compact, finely granu-

FIG. 3. Lamina propria showing two adjacent PAS-positive granule containing cells and a third cell that may be a plasma cell (top left). Representative granules (Gran) show their content of "bacillary bodies" (BB) in various states. Intracellular fat droplets (F) are also observed. Uranyl acetate stain. 24,000 ×.

FIG. 4a. Jejunal epithelial cell showing brush border composed of normal microvilli (Mv) and the rarely seen intraepithelial content of "bacillary bodies" (BB). Lead hydroxide stain. 19,000 ×.

FIG. 4b. Details of "bacillary bodies" seen in Figure 4a. Their membranes and vacuolated granular contents are apparent. 35,000 ×.

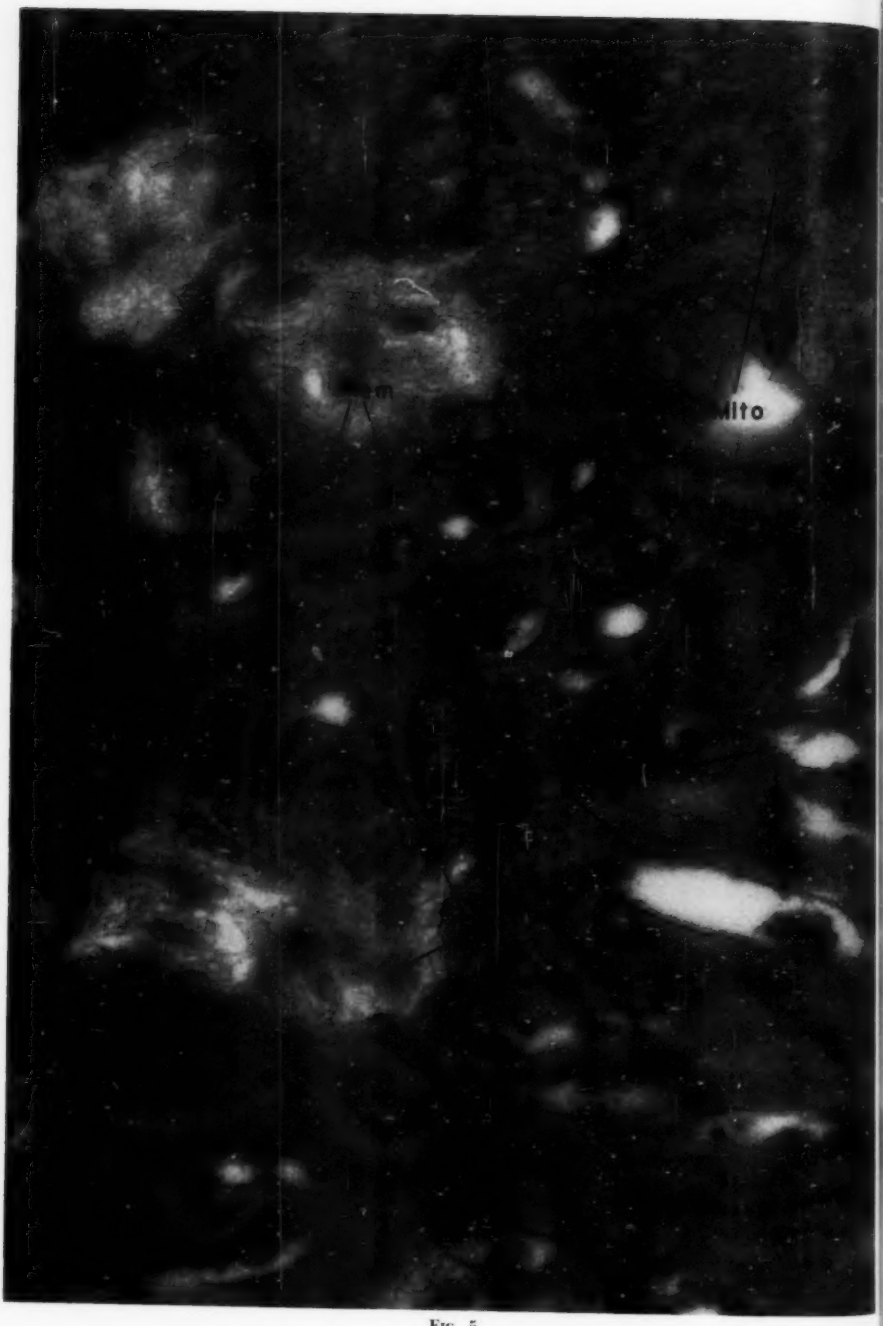


FIG. 5

lar clusters of Gram positive material in the lamina propria (Fig. 11). The tissue had been fixed immediately and the overlying mucosa was intact. Since the same masses are PAS positive and reveal individual rod-shaped bodies, they no doubt represent extracellular "bacillary bodies" also. Giemsa staining brings out the same clusters nicely as moderately dark, blue masses (Fig. 12). Acid-fast and Machiavello stains are negative on the same specimens. Intracellular granules are not stained by either the Giemsa or bacterial stains. It is important to note that post mortem autolysis usually makes interpretation of these stains difficult in autopsy tissue.

Silver Staining Techniques

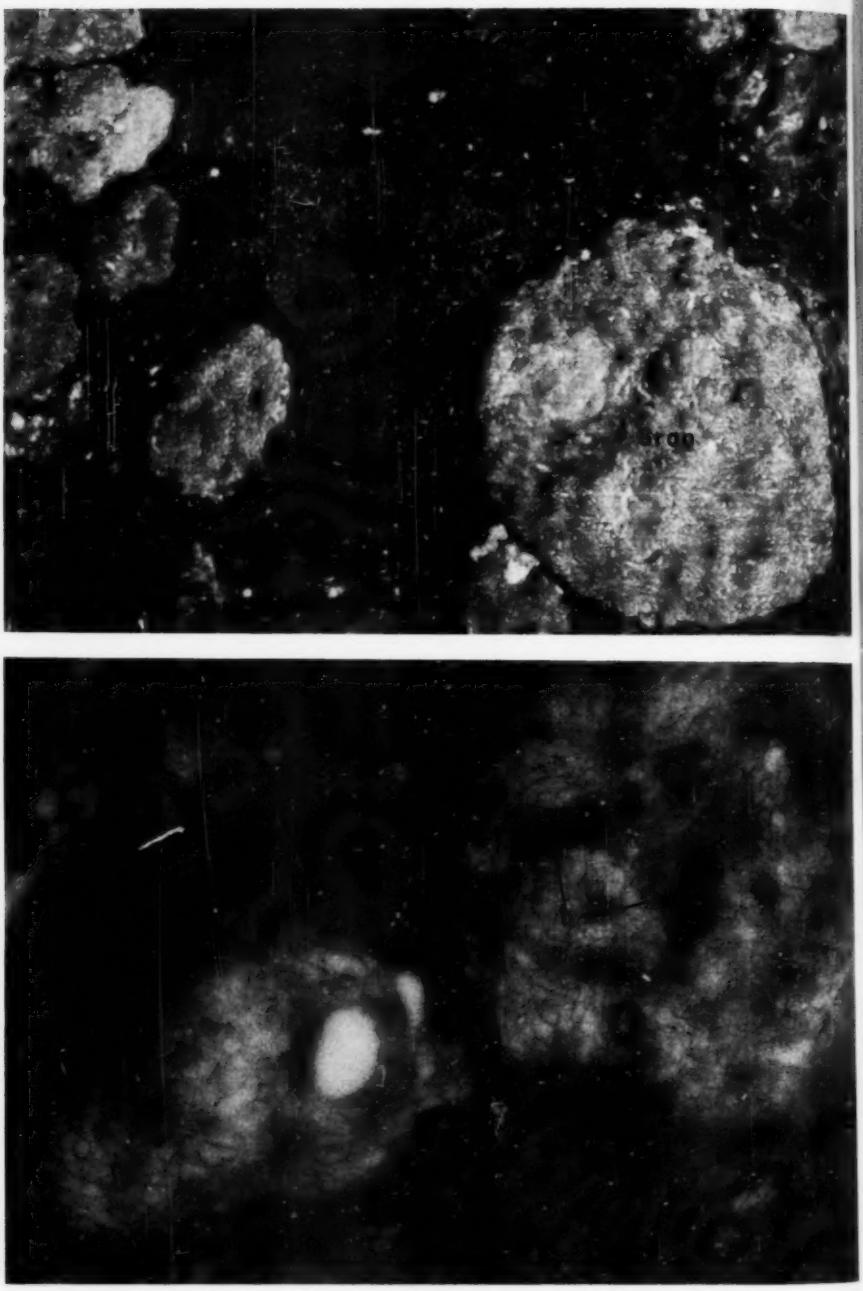
The periodic acid-methenamine silver and Gomori silver reticulin stains give results similar to those with the PAS stain. They can be useful for demonstrating extracellular bacillary bodies in very thin (1 micron) sections, but like the PAS stain, the methenamine silver and Gomori staining reactions do not distingiush well between "bacillary bodies" (either extracellular or intracellular) and intracellular *granules*. However the Levaditi technique stains only a few intracellular granules strongly and in addition occasionally shows some very small rod-shaped bodies intracellularly and extracellularly (Fig. 13). These could well be the "bacillary bodies". The Warthin-Starry spirochete stain showed no structure of note resembling "bacillary bodies".

DISCUSSION

It is clear from other reports that many of the electron microscopic findings outlined above are not unique to this case of Whipple's disease. Haubrich, *et al.* (4) observed that the intracellular PAS-positive granules consist of "—clusters of minute, vacuolated bodies.", and those authors also saw extracellular structures that may be very similar to the "bacillary bodies" in the present case. Cohen, *et al.* (6) have described a case of Whipple's disease in which strikingly similar looking extracellular bodies were found along with large inclusions composed of filamentous membranes corresponding to the PAS positive granules.

The interpretation of electron microscopic findings in Whipple's dis-

FIG. 5. Details of the PAS-positive granules seen in Figure 3. The granules have a definite outer membrane (Mem) that is unlike the endoplasmic reticulum (ER); the granules are also different from the mitochondria (Mito). In many instances the intragranular "bacillary bodies" (BB) still closely resemble those seen extracellularly while elsewhere only vague smudges (? BB) suggest their presence. The intragranular network of membranes is more apparent in those granules which contain less easily recognizable "bacillary bodies". Uranyl acetate stain. 49,000 ×.



FIGS. 6 AND 7

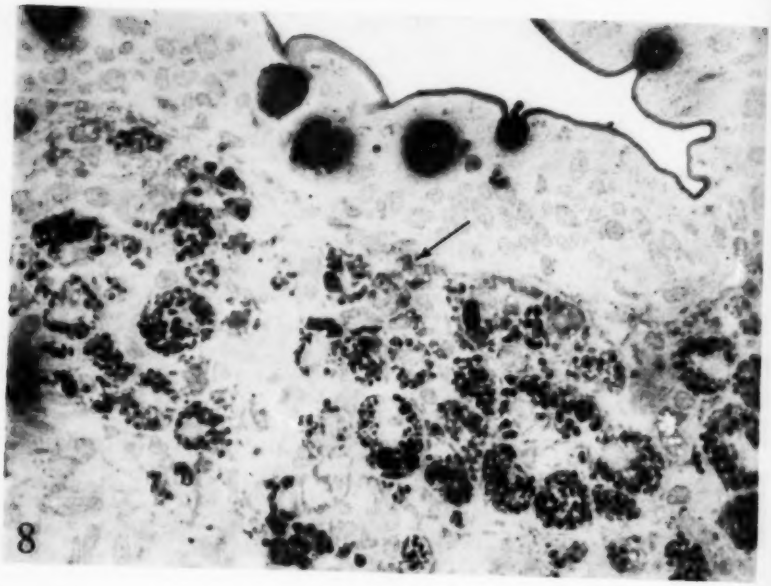
ease is quite variable however. Haubrich, *et al.* (4) suggest that the extracellular structures seen by them may represent a cell product extruded from the PAS-positive granules and that the extracellular bodies and granules are an abnormal protein-carbohydrate complex elaborated by the cell. On the other hand, Cohen *et al.* (6) consider the "ovoid bodies" seen extracellularly in their case to be of possible viral origin. Those authors make no specific observations as to how their extracellular bodies relate to the PAS-positive granules although they do show an "ovoid body" intracellularly. Our conclusions from the present case regarding the nature of very similar extracellular bodies are somewhat at variance with both suggestions. The present findings are thought to indicate that the "bacillary bodies" most closely resemble microorganisms of a bacterial type, and that genesis of the PAS-positive granules may be directly related to the "bacillary bodies".

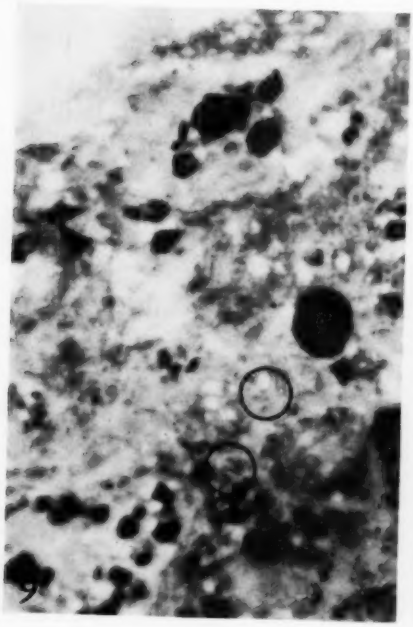
At least two observations make untenable the suggestion that the "bacillary bodies" are virus particles. The first concerns their size. The largest viruses described so far, such as those of the psittacosis group, measure only about 400 millimicra in greatest dimension; most types are much smaller. The "bacillary bodies" are often at least one micron long even in ultrathin sections. Secondly, their ultrastructural morphology, consisting of an outer jacket, membranes and a variable internal architecture, is more complex than that described so far for the viruses (12). On the basis of size and ultrastructure the "bacillary bodies" might certainly be rickettsial, particularly since those organisms have an ultrastructure pattern similar to that for bacteria. On the other hand, the occurrence of "bacillary bodies" in large numbers extracellularly and failure to take the Machiavello stain is unlike the Rickettsiae.

In fact all major electron microscopic features of the "bacillary bodies" correspond to similar structures that have been described in thin section studies of known bacteria (12, 13). The "bacillary body's" outer jacket and limiting membranes correspond to various components of a bacillus cell wall and plasma membrane. The granular material noted within the "bacillary body" duplicates a bacterial cytoplasm; in known bacteria this zone contains large amounts of ribonucleoprotein. The central less

FIG. 6. Overall view of a cell containing several PAS-positive granules. There are no definite "bacillary bodies" seen in this picture. The largest granule (Gran) has a diameter greater than that of the nucleus (Nucl). Uranyl acetate stain. 10,000 ×.

FIG. 7. Granule detail from a cell like that shown in Figure 6. Note the delicate network of lines (membranes). There is one structure (a) resembling a "bacillary body" while other dark smudges (b) may be remnants of the "bacillary bodies". Uranyl acetate stain. 54,000 ×.





FIGS. 8 TO 10

dense region found in the "bacillary bodies" from our case of Whipple's disease suggests the "nucleus" noted in some bacterial types.

It would be premature on the basis of the present limited information to seriously consider what specific *type* of bacterium the "bacillary body" may be. Nevertheless, it may be useful to point out that some bacteria certainly fall within the size range noted for the "bacillary bodies", such as some Brucella and Pasteurella organisms and the bacteroides, the latter being common in the intestinal tract. The definite outer jacket renders it unlikely that the "bacillary bodies" are pleuro-pneumonia-like organisms in their presently known form; the absence of a cell wall is one of their major characteristics (14). That the "bacillary bodies" seem to stain in this case as Gram positive organisms is a somewhat curious finding; organisms with their form, size and location are ordinarily Gram negative, as are all of the organisms mentioned above.

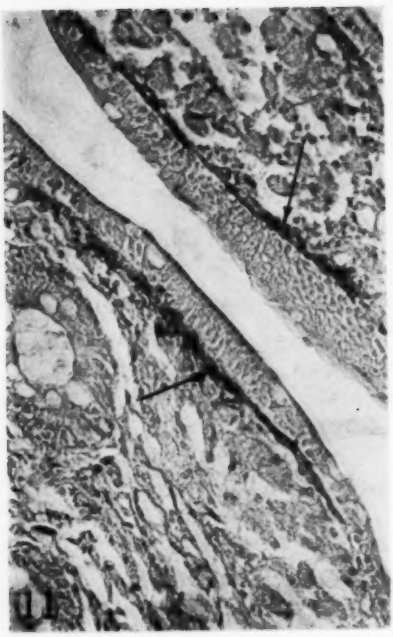
The PAS staining of the "bacillary bodies" seen in our case is also an unusual property; it has been described as a distinguishing characteristic mainly of fungi amongst the micro-organisms. On the other hand, only fungi and certain of the actinomyces amongst the lowest forms of plant life have regularly demonstrated the more complex intracellular organization suggested by the finding of definite mitochondria, internal membrane systems, and a membrane-delineated nucleus (15, 16, 17, 18). The failure to demonstrate such internal components in the present "bacillary bodies", therefore, correlates with bacterial rather than mycotic ultrastructure. The present PAS staining could result from an alteration in the "bacillary bodies" by the host, perhaps by coating it with a secretion. Yet even more intriguing, especially with regard to the origin of the granule, is the possibility that mucopolysaccharides or other substances naturally present in the outer jacket of the "bacillary body" are responsible for this PAS staining.

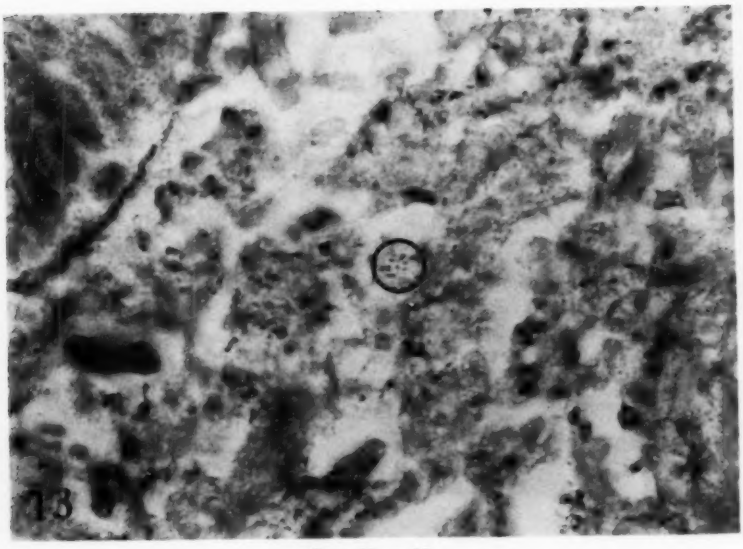
Most authors consider the granule containing cells to be histiocytes and allude to them as such. There is much to be said for this view. Their

FIG. 8. PAS stained, osmic acid fixed 1 micron section of the type used for correlative electron microscopy. The granules in many of the cells are large and deeply stained, others are small and not so deeply stained. Many of the cells in this field are surrounded by PAS-positive masses of "bacillary bodies" (arrow). The epithelium with its goblet cells and brush border are seen at the top of the photograph. 800 ×.

FIG. 9. Swarms of small, extracellular PAS-positive rod-shaped bodies (circles) in the lamina propria. These were seen to be "bacillary bodies" in the electron microscope. Osmic acid fixation, methacrylate "thick" section (about 1 micron). 2000 ×.

FIG. 10. Many PAS-positive rod-shaped bodies (circle) in the lamina propria. These are presumed to be "bacillary bodies" although there was, of course, no confirmation in the electron microscope as in Figure 9. Zenker-formol fixation, standard section. 2000 ×.





FIGS. 11 TO 13

form, distribution and presumed phagocytic capacity are features usually ascribed to histiocytes. Certainly nothing is seen in the present ultrastructure studies that is against the granule-containing cells being histiocytes.

Many theories for the cause of Whipple's disease have been discussed or suggested, but no doubt the most widely held current concept is that it has an endogenous cause, perhaps with a heritable origin or propensity (3, 4, 5, 19). Much stress is usually laid upon the possibility that the peculiar PAS-positive material may be an abnormal product of cell metabolism. If this approach is correct, then the "bacillary body" (assuming it is a micro-organism) might be saprophytic or simply a secondary invader, and the occurrence of "bacillary bodies" intracellularly and within the PAS-positive granules would, therefore, be fortuitous.

Yet the "bacillary body" could have a more fundamental significance in the pathogenesis or etiology of Whipple's disease. Though proof is lacking that extracellular "bacillary bodies" are phagocytized by the granule-containing cells, our thesis regarding origin of the granules assumes this to be the case. It is further believed that the various PAS-positive granule forms may represent a true transformation sequence beginning with those granules that enclose clearly recognizable "bacillary bodies". Their gradual disappearance to be replaced by a granule consisting of a peculiar network of lines or membranes (a form that defies any simple interpretation by current electron microscopy criteria) may be a digestive process which leaves behind the PAS-positive material. The latter, most probably one or more polysaccharide or glycoprotein substances, may be: 1) derived from the "bacillary body", in particular its outer jacket, 2) produced by the host cell as a *normal* reaction to the "bacillary body", or 3) the product or result of an *abnormal* host response to the "bacillary body".

If an abnormal host response to the "bacillary body" exists as a factor in Whipple's disease, it might mean that "bacillary bodies" produce Whipple's disease only in certain individuals. Such an abnormality might indeed be heritable. As a specific example of a *hypothetical* host abnormality in Whipple's disease, the host histiocytes might be deficient in a

FIG. 11. Clusters of Gram positive material are seen in the lamina propria just beneath the epithelium (arrows). Comparative PAS stains suggest that these are "bacillary bodies". Brown and Brenn stain. 287 ×.

FIG. 12. Swarms of faintly seen rod-shaped bodies (circle) in a field comparable to that in Figures 10 and 11. There are also scattered polymorphonuclear leukocytes. Giemsa stain. 1150 ×.

FIG. 13. Scattered argyrophilic rod-shaped bodies (circle) both intra- and extracellularly as seen in the Levaditi stain. Intracellular granules do not usually stain. 1150 ×.

particular enzyme. This enzyme could be of a type that in the normal individual digests one or more PAS-positive substances derived from "bacillary bodies". The hypothetical enzyme might play a role in host's defense against the "bacillary body". Also, undigested PAS-positive substances could accumulate in the histiocytes of an enzyme-deficient Whipple's disease patient, thus leading to the picture of a "storage" disorder.

In addition to the above studies on jejunal tissue, we have studied by conventional microscopy mesenteric lymph nodes and liver from the present patient in a search for structures that might be "bacillary bodies". There was staining of structures suggesting "bacillary bodies" by the Giemsa method in a mesenteric lymph node, and in another node rod-shaped bodies were demonstrated with the Gomori silver reticulin stain. The question of whether or not "bacillary bodies" reside in other tissues, possibly a critical point in determining their significance for Whipple's disease, will no doubt be settled best from a morphologic standpoint by electron microscopy.

As was observed previously (20), it is of great interest that in his original report of this disease (2), Whipple discussed in detail the finding of many very small, rod-shaped structures in a lymph node. Only the Levaditi stain amongst those tried by Whipple demonstrated the rod-shaped bodies satisfactorily. They were most prominent within certain of the large fat accumulations, but were also seen in the "foamy cells" that we now know to correspond to the PAS-positive granule containing cell. Whipple concluded that the rod-shaped structures seen by him might well represent a micro-organism and suggested that they could also be the etiology of the disease. Their similarity to the "bacillary bodies" is, therefore, startling in several respects, including their presumed argyrophilia.

There is no need to belabor the obvious necessity for acceptable microbiological proof of the nature and relevance of "bacillary bodies". However, properly controlled studies directed towards this end would certainly include therapeutic trials using antibiotics. There is no completely convincing evidence that any therapeutic regimen used to date has been more than temporarily effective, but in a recently reported case (21) results with small doses of oral antibiotics seemed to give strikingly favorable results. The same authors discuss the varied experience in the literature with antibiotic therapy in Whipple's disease. Also, in a review of twelve cases (22) it is noted that antibiotics often seemed efficacious, and that the effect was enhanced when combined with ACTH or corticosteroid therapy. Of course, it is always difficult to evaluate therapeutic results in Whipple's disease in view of the well-known occurrence of spontaneous remissions.

SUMMARY

Jejunal tissue obtained by peroral biopsy from a case of Whipple's disease has been studied in the electron microscope and the results are compared with light microscopy findings.

Observations are presented which highly suggest that certain peculiar rod-shaped bodies seen extracellularly and intracellularly are bacterial, and they are therefore termed "bacillary bodies".

Evidence is given that the PAS-positive intracellular granules characteristic of Whipple's disease are derived from the ingested "bacillary bodies" which are themselves PAS-positive.

It is concluded from these findings that Whipple's disease may be an unusual infection.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

May 14, 1961

QUANTITATIVE ESTIMATIONS OF NUCLEOPROTEIN-REACTIVE GAMMA GLOBULINS AND SERUM COMPLEMENT IN SYSTEMIC LUPUS ERYTHEMATOSUS

A. S. Townes and A. G. Osler

A precise and reproducible procedure has been developed for quantitative estimation of the serum gamma globulins that react with nucleoprotein. This report presented the method and results obtained in systemic lupus erythematosus (SLE) and related disorders, some preliminary characterization of the reactants, and finally correlations of nucleoprotein-reactive gamma globulins with clinical findings and serum complement.

The method is based on the use of a quantitative complement fixation technique for the assay of human gamma globulin as antigen, and thus may be applicable to the study of other autoimmune phenomena. A standard quantity of insoluble strands of calf thymus nucleoprotein in a finely particulate suspension was reacted with appropriate dilutions of heat inactivated test serum. Nucleoprotein particles were then recovered by centrifugation, washed to remove unbound serum proteins and dispersed in alkaline buffer to minimize aggregation. An aliquot of this nucleoprotein-gamma globulin complex was then used as antigen in a quantitative complement fixation reaction with 3.0 μg. rabbit antihuman gamma globulin N and 100 $C'H_{50}$ guinea pig complement. The number of units of complement fixed in this reaction was converted to microgram equivalents of gamma globulin nitrogen by interpolation on a calibration curve established simultaneously under identical conditions using standardized increments of human gamma globulin.

Thirty normal sera gave nucleoprotein-reactive gamma globulin values of 0.5 to 3 μg. N per ml. Sera from 35 patients with SLE gave values from 5 to 60 μg. N per ml. with 27 patients having more than 15 μg. N per ml. Data were presented to show that in general higher levels were associated with active disease with decline following improvement on steroid therapy. Most sera having more than 10 μg. N per ml. of nucleo-protein-reactive gamma globulin also gave positive LE cell tests. Occasional exceptions, however, and other data suggested that the nucleoprotein suspension contained multiple antigens reactive with antinuclear factors in SLE serum.

Six of 57 patients with a variety of other diseases were found to have nucleoprotein-reactive gamma globulin slightly above the normal range. A significant number of abnormal reactions was observed in sera from patients with rheumatoid arthritis, scleroderma, liver disease, and chronic biologic false positive serologic tests for syphilis. In general values were lower than those in SLE. Tests with some of the sera from patients with rheumatoid arthritis and liver disease indicated that this reactivity may be associated predominantly with a macroglobulin in contrast to SLE.

An increment in nucleoprotein-reactive gamma globulin in fresh serum as compared to heat inactivated serum both in normal and abnormal sera was consistently demonstrated. Data were presented to indicate that this increment of gamma globulin was

dependent upon the integrity of serum complement (or possibly only the first component). The data did not suggest the presence of a heat labile antibody.

Low serum complement values were found on one or more occasions in most patients with SLE and in all of those with renal involvement. An inverse correlation of serum complement with levels of nucleoprotein-reactive gamma globulin and disease activity was demonstrated. The significance of these correlations and possible implications of these changes in the pathogenesis of the renal lesion in SLE were discussed.

THE DEVELOPMENTAL GENETICS OF MELANOCYTE DIFFERENTIATION ANALYZED WITH THE ELECTRON MICROSCOPE

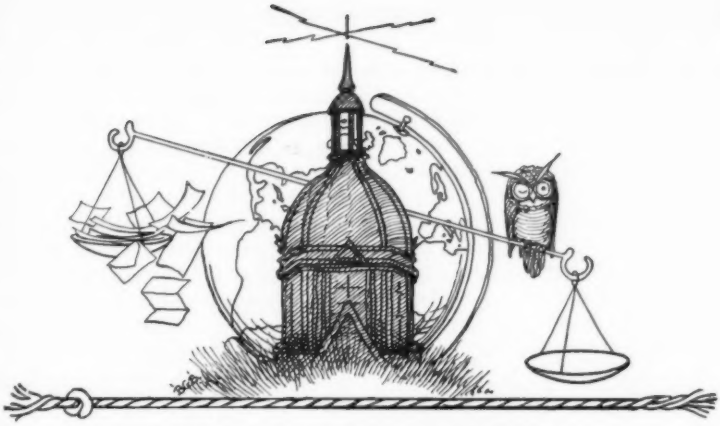
FRANK H. MOYER

The structure of melanin granules in the house mouse is genetically determined, and the chemical and physiological factors in melanin production have been intensively investigated. Electron microscope observations show that melanin granules have a characteristic fine structure. Hence differentiating melanocytes in inbred strains of mice carrying different mutant genes affecting pigmentation afford excellent material for the study of genetic effects on cell fine structure during cytodifferentiation. Accordingly differentiating melanocytes in various inbred strains of mice were examined with the electron microscope. The origin of melanin granules was determined and a sequence of fine structural changes during their development was noted. Mutant genes were observed to affect these factors. These observations were compared to similar observations on chick retina and mouse melanoma.

Melanin granules appear to form by the orderly aggregation of thin fibers in parallel array. A moderately dense material is deposited on the fibers as they aggregate. The deposition of a material intrinsically opaque to the electron beam (melanin) follows until the underlying fine structure is obscured in the mature melanin granule. The developmental sequence is essentially the same in the chick, but differs slightly in melanoma.

The thin fibers of early melanin granules are often observed to be in contact with small dense particles approximately 150 Ångstrom units in diameter which stain intensely with uranyl acetate and lead subacetate. Moreover the fibers exhibit an axial periodicity which varies in accordance with mutant alleles at the "B" and "C" loci in the mouse. These observations suggest that the fibers are aggregates of several protein components synthesized on the ribosomes. Observations of the gross effects of genes on melanin granule ontogeny support this hypothesis. The gene for brown (at the "B" locus) changes the appearance of the melanin deposited on the fibers from the finely granular, homogeneous wild type (black) to a roughly flocculent mutant type (brown). The gene for albino (at the "C" locus) completely prevents the deposition of melanin on fibers which appear normal in other respects. The gene for pink eye (at the "P" locus) allows deposition of melanin in accordance with the genotype at the "B" locus but prevents the orderly parallel aggregation of the early thin fibers so that the mature granule has a ragged, uneven appearance. Thus the granule contains at least three independently mutable subunits. The other loci affecting pigmentation appear to act indirectly by altering granule size, shape, and distribution.

These observations emphasize the complexity of the gene-product interactions which underlie cellular differentiation.



BOOK REVIEWS

Persuasion and Healing. A Comparative Study of Psychotherapy. By JEROME D. FRANK. 282 pp., $5.50. The Johns Hopkins Press, Baltimore, Md., 1961.

As the title implies, this is an exploration into the healing process through psychotherapy as practiced in its various forms currently, and comparing it with alterations of symptom and attitude achieved by other forms of interpersonal influence, for example, shamanism, religious revivalism, even Communist brain-washing. Implicit in all forms of psychotherapy are certain assumptions, namely, that for people with emotional malaise and psychosomatic symptoms, our culture provides for people who are trained to believe that they have methods at their disposal for their alleviation. Not only do the practitioners believe it but the sufferers believe it. Much of the trappings of modern psychotherapy, even its sectarianism, finally trickle down to both practitioners and recipients of the practice as reinforcements of these basic assumptions. Dr. Frank pointedly remarks on the special position that psychoanalysis has in this country today as lending itself to these assumptions. As a more blunt-spoken analyst told me on one occasion, analysts could no more afford to give up the title of analyst than Buick could afford to give up its title. When he said this, I could not help but remember Buick's slogan: "When better automobiles are built, Buick will build them," and I am sure my blunt-spoken friend has much the same feeling about psychoanalysis.

The author cites the well-known fact that percentages of cures, or improvements through psychotherapy, are notoriously about the same no matter what variety of psychotherapy is employed. He points out that this provides circumstantial evidence that all types of psychotherapy have something in common and that it is this common factor which produces a similarity of results. There is one caution, however, about this, namely, that different schools have different criteria for improvement so that gross figures are not immediately transferable from one group to another. The assumption on the part of the patient that the practitioner has what he needs at his command, he thinks is the reason for immediate symptom improvement, so commonly observed in any method, even the placebo effect.

It is not determined yet to what extent long distance attitudinal changes may or may not differ from one school to another. He points out that the amount of time

spent in achieving attitudinal change would appear to be more closely related to the practitioner's assumptions than those of the patient. In short, practitioners trained in long-term treatment assumptions naturally assume that long-term treatment is necessary.

He noted from an experiment set up at the Hopkins that the long-term changes in patients continued even after short-term psychotherapy. This is a point which needs elaboration.

If I read the author correctly, his general conclusion is that the basic assumption, held by practitioners and by clients, lends itself to the art of persuasion; and not just the obvious kind of persuasive psychotherapy as advocated years ago by Dubois, but in many subtle ways about which the practitioner himself may be, but should not be unaware. As the author points out, if one is to practice healing, it is well to be as conscious of what one is doing as possible. Also, if I read him correctly, he leans rather heavily at this time on the grounds of psychological experimentation to the belief that psychotherapy works as a learning process. This should not come as any great surprise. Many of us long have felt that not only do patients come to psychotherapists because of their belief that the psychotherapist has at his command the knowledge of how to live better but it is incumbent upon the psychotherapist that he actually know how to live better. Else, why is such care exercised in the acceptance of young physicians for training in the art.

This is a most readable and interesting book and while stripping psychotherapy, or certain brands of it anyway, of some of its treasured pretension, it substitutes sensibly acceptable views which should have a uniting influence in a field which has too often been split by fanatical schisms. As a technical piece of writing it is simple but erudite, far-reaching in its scope, and from the reader's standpoint, has a very helpful summary at the end of each chapter together with most useful explanatory notes in an appendix. The book was written while Dr. Frank was enjoying a year's residence at The Center For Advanced Study in The Behavioral Sciences. I would suppose from the breadth of the material in the book, that his contacts at this Center had been most fruitful.

There is an appreciative preface by Dr. John Whitehorn, whose long-time influence Dr. Frank gladly acknowledges.

WENDELL MUNCIE

The Hypothalamus of the Cat. A Cytoarchitectonic Atlas with Horsley-Clarke Coordinates. By RUTH BLEIER. 109 pp., $15.00. The Johns Hopkins Press, Baltimore, Md., 1961.

The Horsley-Clarke stereotaxic instrument is a device so well known for its value in accurately placing electrodes within the depths of the brain, that its image has over-shadowed that of its indispensable partner, the topographic atlas arranged for use with the Horsley-Clarke coordinate system. Experimental neurologists and neurosurgeons who have had spectacular successes with the stereotaxic instrument are keenly aware that the precision of placement of recording, stimulating, or lesion-producing electrodes, inherent in a well-machined stereotaxic instrument, can be fully exploited only by the availability of a carefully prepared atlas based on well-preserved brain specimens. Dr. Bleier has made an important contribution to the armamentarium of experimental neurologists by fulfilling this requirement, especially since her book deals with the favorite species of mammalian neurophysiologists and with a region in which so many important functions are crowded into a surprisingly small portion of the brain.

Although the value of this volume rests primarily upon the topographic atlas, the descriptive aspects are enhanced by a critical review of significant literature dealing with topography, nerve fiber connections, and functions of many of the neuron groups described. Of special interest is her re-analysis of the now classical stimulation work of Hess. Brief anatomical notes on the hypothalamus of some of the larger feline species are also included.

The high standard of neuroanatomical scholarship to be expected from the laboratory of Jerzy E. Rose is matched by the excellence of manufacture of this volume. Photographic illustrations of brain sections are beautifully reproduced on a scale generous enough to define most cell groups with clarity, and to include adjacent regions of dorsal diencephalon and of telencephalon. Although this necessitates a large page size, this inconvenience is more than balanced by the large type face, and generally elegant appearance.

DAVID BODIAN

A Student's Guide to Obstetrics and Gynaecology. By CHRISTOPHER J. DEWHURST. 229 pp., $4.00. J. B. Lippincott Co., Philadelphia, Pa., 1961.

A booklet of this size is always difficult to review. Initially, one feels inclined to be upset at the thought that one's favorite specialty could be condensed into 225 pages of large and very readable print.

This format, however, is achieved simply. All operative obstetrics and gynecology is left out, and in a "guide" this is only logical. Sometimes, however, it is not so much what is left out in such a vade mecum that is worrisome but what is left in. Statements which in a textbook can be toned down with qualifying remarks here sometimes acquire excessive authority by their brevity. Thus, to imply, without ifs and buts, that pelvic mensuration is of no importance since only cephalopelvic disproportion matters, that pethidine 100 mg. may safely be repeated several times during a normal labor, that external version *should* be tried if a breech presentation is not spontaneously turned to vertex by the 34th week, or that severe degrees of placenta previa will almost always be treated by *lower segment cesarean section*, or again, that induction for postmaturity is a good idea, seems like over-simplification that medical students might not question.

The compression of gynecology and endocrinology into 68 pages will be sufficient unto itself to point out that as a guide to gynecology this booklet leaves something to be desired. Preinvasive carcinoma of the cervix is not mentioned at all, for instance. However, before the reader begins to wonder why a reviewer should spend so many words seemingly criticizing this work, when surely one sentence might have been enough to do the job, if that were all one desired to do, the reverse side of the coin should also be mentioned.

The reverse side of the coin is this: The 152 obstetrical pages are studded throughout with so many little pearls of wisdom and warning, that I confess to some envy of the author's ability to bring out so many cardinal obstetrical rules so succinctly and so readably. In the final analysis, the reviewer is left to face an enigma. The enigma is this: Will the medical student be able to realize the value of the numerous little pearls, and will he be able to reject the several possibly dangerous, over-simplifications and omissions? Perhaps this book should have been called "A Guide for the Student's Guide to Obstetrics and Gynaecology."

ANDRE E. HELLEGERS

Medical, Surgical and Gynecological Complications of Pregnancy. By the Staff of the Mount Sinai Hospital, New York City. Edited by ALAN F. GUTTMACHER and JOSEPH J. ROVINSKY. 619 pp., $16.50. The Williams & Wilkins Co., Baltimore, Md.

Pregnancy is a normal biological process that is bound to occur in the lives of large numbers of women. Because it is a common event in the lives of women, pregnancy is bound to be superimposed upon almost the whole range of pathological states afflicting women under fifty years of age. It is strange, therefore, that until Alan Guttmacher went to the Mount Sinai Hospital in 1952 no one had ever conceived of establishing a whole host of interdisciplinary clinics for the management of the non-obstetrical complications of pregnancy. Now, seven years later, the members of these ten special clinics have harvested the fruits of this combined knowledge and experience. The resulting book will fill a space too long empty on the bookshelves of every physician who is ever called upon to see a pregnant woman. Here, at long last, in one book virtually every non-obstetrical illness that may occur in conjunction with pregnancy is discussed, usually at length. Some sixty-nine contributors from the active staff of Mount Sinai Hospital have reviewed the relationship of every system of the body to pregnancy. The normal physiology of these systems in pregnancy is discussed. The emphasis, however, is upon pathologic states and their therapy. Especial emphasis is put upon the possible application of sterilization and abortion, operations which are much less often indicated than was previously thought. One may disagree here and there with the therapy recommended. However, this book is a must for every physician except those who deal only with men or exclusively with patients over fifty.

GEORGE W. CORNER, JR.

Orthopaedia. By NICOLAS ANDRY. Facsimile Reproduction of the First Edition in English, London, 1743. Published by J. B. Lippincott Company, Philadelphia and Montreal, by a photo-offset technique of the 1743 English Edition which was located in the library of the Yale School of Medicine. Introduction by Dr. Anthony F. DePalma. This two volume replica was sponsored by the Association of Bone and Joint Surgeons. The full title of the book is:

> Orthopaedia: Or, the Art of Correcting and Preventing Deformities in Children:
>
> > By such means as may easily be put in practice by parents themselves and all such as are employed in education of children.
> >
> > To which is added,
> >
> > A defense of the Orthopaedia by way of Supplement by the Author.

This book was translated from the French of M. Andry, Professor of Medicine in the Royal College, and Senior Dean of the Faculty of Physick at Paris.
The French edition was published in 1742. One year later Andry died and the English translation appeared in London in 1743.

In the preface, the author explains the title of the book and points out that he has formed this word of two Greek words, Ortho—meaning straight or free from deformity, and the second part of the word coming from the Greek origin of one word, child. He compounded this word in order "to express in one term the design I propose which is to teach the different methods of preventing and correcting the deformities of children."

As Dr. DePalma states, "To the orthopaedic surgeon, Andry is more than the coiner of a word. His book contains fundamental information on such entities as curvature

of the spine, club feet, congenital dislocations including the hip, bow-legs, and congenital deformities. Even more important, he was the first to note the active participation of muscles in producing deformities of the skeletal system. . . . His work with the muscular apparatus influenced many orthopaedic surgeons who came after him, especially Venel, Delpech, Ling and, to some degree, Hunter and Lane."

On reviewing these two volumes one sees that Andry did not confine himself to the musculoskeletal system but described various deformities of the skin and their treatment, cleft palate, hair lip, prolapse of the anus in infancy and, in general, all deformities that were externally observed. He also discussed in great detail the rôle of the nurse and one realizes that at the time this book was written many people felt that deformities not only of the body of the child but even of the mental attitude of the child depended on the care that he received from his nurse. One is also aware of the great rôle which the wet nurse played in the bringing up of many children at that time before the day of adequate artificial formulae for infants. One may be amused perhaps that the deformities supposedly due to rickets were blamed on the way the children were wrapped in swaddling clothes at the time this book was written, but one must also realize that the author recognized from empiric evidence that the form of a child could be changed by splinting as he grew. He did not know, for instance, about the epiphyseal growth mechanism and the possibility of altering the direction in which these growth areas would proliferate in relation to the long axis of the body by splinting externally but he did know that splints could be effective and he goes into some detail as to the way in which this should be done. This is discussed in relation to a figure which one finds opposite to page 211 in the first volume.

In this book, Nicolas Andry planted the seed of the surgical specialty of orthopaedics. Orthopaedic surgery in general has become interested in the correction and prevention of deformities not only in children but in adults. Today those who specialize in orthopaedic surgery are interested in careful correction of fresh deformities caused by injury as in the case of displaced fractures and in the deformities of genetic origin and in the general field of rehabilitation in which one not only tries to prevent or correct a deformity, but return the patient to economic independence in the society in which he lives. Nevertheless, these books constitute the first outline of the field which has received its name from Nicolas Andry and it is therefore of immense interest to orthopaedic surgeons in general to at least read this source book of their specialty.

ROBERT A. ROBINSON

Current Therapy—1961. Edited by HOWARD F. CONN. 806 pp., $12.50. W. B. Saunders Co., Philadelphia, Pa.

The current (thirteenth) volume of this annual publication continues the high standards previous issues have established. While almost identical in format to previous editions, the 1961 volume contains 82 per cent of its 307 articles as entirely new treatises on their respective topics, continuing Dr. Conn's policy of having most of the topics covered by a new writer in each issue. The real value of this policy is that the reader, by consulting two or three recent volumes, obtains an excellent survey of current therapeutic approaches to the problem involved. This is not to imply that the single volume is not of value, for to the contrary, the list of consulting editors and contributors is composed of men of the highest professional qualification and experience, and their coverage of the subjects includes all of the measures of well established merit as well as of newer measures with discussion of their value or potential risk.

The internist will find this volume a very valuable reference, and it should be found in every house staff library.

ROBERT E. MASON

Blood Diseases of Infancy and Childhood. By CARL H. SMITH. 572 pp., $17.00. The C. V. Mosby Co., St. Louis, Mo., 1960.

With the publication of this book, persons interested in blood diseases of infants and children now have available for the first time a comprehensive and instructive text. As Dr. Smith states in the preface, emphasis is placed on the "interpretation, diagnosis, and management of the blood diseases encountered in pediatric practice." Consequently, special consideration is given to the etiology and treatment of hyperbilirubinemia of the newborn, as well as other abnormalities of the blood which occur exclusively in the neonatal period. These chapters are especially valuable since many of the topics are too briefly presented in general textbooks of hematology.

The bibliography for each chapter is comprehensive, but not exhaustive, and most of the pertinent references in the recent literature are listed. Since the diagnostic and therapeutic aspects of the pediatric blood dyscrasias are emphasized, it is important to point out that Dr. Smith and his group have had extensive clinical experience in this field, and in many instances have contributed original observations relating to the clinical management of these problems. One minor criticism is the paucity of illustrations, notably the graphic presentation of relevant case histories. Further editions of this important book would be enhanced by the inclusion of this material.

This book is highly recommended to all persons caring for children with hematological diseases.

WILLIAM H. ZINKHAM

Clinical Applications of Cardiopulmonary Physiology. By M. HENRY WILLIAMS, JR. 233 pp., $7.50. Paul B. Hoeber, Inc., Medical Division of Harper & Bros., New York, N. Y., 1960.

The author states in the preface "It is the intent of this book to outline normal cardiopulmonary physiology, to point out the areas that may be assessed by physiological techniques, and to indicate the bearing that such studies have on the diagnosis, evaluation, and treatment of patients with cardiopulmonary disease." In this small volume he has achieved his aim remarkably well.

The first five chapters are devoted to cardiopulmonary physiology and a discussion of heart disease from a physiological viewpoint. The second five chapters include discussions of pulmonary disorders, also weighted by a physiological approach. Three chapters are concerned with hypoxia, dyspnea and muscular exercise. A brief discussion of methods of measurement of cardiopulmonary function appears in the last chapter.

The outstanding features of this book are the excellent diagrammatic illustrations and the lucid presentation of complicated material. The book is pleasantly readable in part because names and references to the original work have been omitted from the text. The technique of including references divided into subject at the end of each chapter permits the reader easy access to more detailed explanations if he seeks them. The references include the outstanding recent contributions on each subject, the majority of them written in the past ten years.

The practicing physician, house officer, and medical student will profit most from this work. The student of cardiopulmonary diseases might resist the simplifications

which were necessary in a text of this size, although he will find in the organization of the text and references a useful guide.

MARY ELLEN AVERY

Physiology of Prematurity. Transactions of the Fourth Conference March, 1959. Edited by JONATHAN T. LANMAN. 187 pp., $4.50. The Josiah Macy, Jr. Foundation Publications, New York, N. Y., 1960.

This volume deals with the heat and the biochemical regulation of the newborn infant. The material discussed is basic to an understanding of the mature as well as the premature infant. This book, therefore, while of interest primarily to the research worker, has a meassage for the clinician as well. The message is that the newborn infant's regularatory mechanisms have a narrow range of safety. Left in its natural surroundings the normal newborn is able to do all that is asked of it, but if we need to interfere to correct abnormalities we must do this with great care lest we worsen the situation rather than reestablish homeostasis.

GEORGE W. CORNER, JR.

An Outline of Chemical Genetics. By BERNARD S. STRAUSS. 188 pp., $5.00. W. B. Saunders Co., Philadelphia, Pa., 1960.

During the past fifteen years there have been and continue to be a number of extraordinary developments in the field of genetics. Taken together these developments may ultimately have more importance to the understanding of human variation and disease than the sum of all other present achievements in medicine. A spate of books have now appeared with the intended purpose of introducing the uninitiated reader to the experiments of modern genetics. The most skillfully done general account is presented in this book by Dr. Strauss. As a microbiologist Dr. Strauss is particularly well fitted to describe the "new genetics" much of which has resulted from the study of micro-organisms. The non-geneticist should understand however, that it is the author's thesis (and bedrock belief of geneticists) that the principles discovered in micro-organisms have validity—on the whole—for man.

Presentation devolves about four general concepts and their consequences. These concepts concern, firstly, proteins as gene products, secondly, identity of the hereditary material with desoxyribonucleic acid (DNA), thirdly, the creation of new combinations of hereditary units as a result of their linear arrangements, and fourthly, mutations as errors in DNA structure. These concepts are found in all the new books. What distinguishes this book is its clarity and the thoughtfulness with which these concepts are interrelated. Cumulation takes the form of two chapters, one on nucleocytoplasmic relationships and the problem of protein synthesis and the other on biochemical genetics in man. This work is not a compendium of human errors of metabolism rather it describes the foundation without which the ultimate importance of modern genetics to medicine cannot be appreciated.

SAMUEL BOYER

BOOKS RECEIVED FOR REVIEW

Annual Review of Medicine, Vol. 12, 1961. Edited by DAVID A. RYTAND. 455 pp., $7.00. Annual Reviews, Palo Alto, Cal.

An Atlas of Acquired Diseases of the Heart and Great Vessels. By JESSE E. EDWARDS. Set

of 3 volumes $70.00. Vol. I: Diseases of the Valves and Pericardium, 520 pp. Vol. II: Coronary Arterial Disease, Systemic Hypertension, Myocardiopathies, the Heart in Systemic Disease, and Cor Pulmonale, Acute and Chronic, 1020 pp. Vol. III: Diseases of the Great Vessels, 1437 pp. W. B. Saunders Co., Philadelphia, Pa., 1961.

Cardiovascular Abstracts I—1960. Selected from World Literature. American Heart Association Monograph. Edited by STANFORD WESSLER. 192 pp. Published by the American Heart Association, Inc., New York, N. Y., 1961.

Clinical Disturbances of Renal Function. By ABRAHAM G. WHITE. 468 pp., $10.50. W. B. Saunders Co., Philadelphia, Pa., 1961.

Diagnostic Cytology and Its Histopathologic Bases. By LEOPOLD G. KOSS in association with GRACE R. DURFEE. 380 pp. $16.50. J. B. Lippincott Co., Philadelphia, Pa., 1961.

Disease and Injury. Edited by LEOPOLD BRAHDY. 482 pp., $12.50. J. B. Lippincott Co., Philadelphia, Pa., 1961.

A Manual of Cutaneous Medicine. By DONALD M. PILLSBURY, WALTER B. SHELLEY and ALBERT M. KLIGMAN. 430 pp., $9.50. W. B. Saunders Co., Philadelphia, Pa., 1961.

Principles of Dynamic Psychiatry. Including an Integrative Approach to Experimental and Clinical Psychology, 2nd edition. By JULES H. MASSERMAN. 332 pp., $8.00. W. B. Saunders Co., Philadelphia, Pa., 1961.

Symposium on Coronary Heart Disease. American Heart Association Monograph, Number Two. Edited by HERRMAN L. BLUMGART. 154 pp. Published by the American Heart Association, Inc., New York, N. Y., 1961.

IMPROVING FLAME PHOTOMETER STABILITY[1]

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Commercial internal standard flame photometers are designed primarily for the determination of sodium and potassium ion concentrations in diluted serum. Certain design compromises have been made to achieve optimum stability and sensitivity in that concentration range. When such instruments are used for determinations in which the ion concentrations must of necessity be considerably lower, performance suffers from loss of sensitivity, stability, or both. A typical example of this problem is the determination of the sodium content of small muscle samples.

The modifications described here pertain to the Baird-Atomic DB-4 flame photometer, but should be equally applicable to other photometers of similar design. The objective was threefold: 1. To increase sensitivity. 2. To minimize common-mode variations; that is to say, variables such as gas pressure which affect both the sodium (or potassium) ion signal and the lithium ion signal. 3. To minimize the response to such common-mode variations which remain. Modifications were limited to those which involved no major mechnical, optical, or electrical alterations.

SENSITIVITY

There are two types of sensitivity to be considered in flame photometer operation. The first is the sensitivity of the measuring potentiometer in detecting changes in concentration in terms of dial divisions per percentage change in concentration. The second is the sensitivity of the galvanometer in detecting error in the measuring dial setting in terms of galvanometer divisions per measuring dial division. This sensitivity of the galvanometer as a null sensing device might better be termed readability. The major concern when dealing with low sodium (or potassium) ion concentrations is the sensitivity of the measuring potentiometer.

The sensitivity control is an attenuator of the sodium (or potassium) signal. When the attenuation is minimum, no further increase in electrical sensitivity is possible except by amplification. However, the instrument measures the ratio of the sodium signal (which is a non-linear function of

[1] Supported in part by grant B-894 (C4) from the Division of Neurologic Diseases and Blindness, National Institutes of Health, U.S.P.H.S. and by a contract with the U. S. Army Chemical Corps.

the sodium concentration) to the lithium signal (which is a non-linear function of the lithium concentration). By reducing the concentration of lithium added to standards and unknowns, the sensitivity to changes in the sodium (or potassium) concentration can be increased.

This method of increasing the sensitivity of measurement is limited by the fact that reducing the lithium signal reduces the sensitivity of the galvanometer in detecting unbalance. This latter does not reduce accuracy until the operator's "dead-band" begins to be increased, where "dead-band" may be considered to be the number of dial divisions required to move the galvanometer perceptibly. Thus, reductions in galvanometer sensitivity do not contribute to inaccuracy until the operator is unable to perceive galvanometer movements in response to say ± 5 dial divisions (± 0.5 per cent full scale).

STABILITY

Increased sensitivity usually makes instability more readily apparent. Thus, increased sensitivity without a similar increase in stability is of little value. Attention was, therefore, turned to improving stability.

While the internal standard flame photometer is much less susceptible to changes in the flame and changes in vaporization than the direct instrument, it is by no means insensitive to changes in these parameters. One approach to the problem was to reduce fluctuations in these parameters to a minimum.

Flame Stability. Replacing the customary one stage air pressure regulator with a two stage regulator and the installation of a two stage gas pressure regulator reduced both short and long term variations in flame intensity.

Vaporization Stability. Constancy of the concentration of the nebulized and vaporized solution delivered to the flame is as important as stability of the flame itself. The practice of rinsing the funnel with water between samples prevents contamination due to evaporation, but may prevent achieving a steady-state concentration of ion at the flame. Silicone coating the funnel and nebulizer eliminates the necessity of rinsing.

The accumulation of a drop of solution on the nebulizer tip produces a transient galvanometer deflexion when it is finally blown off. Occasionally, the drop will "jitter" on the tip and produce a more protracted and less obvious error. A modest reduction in the air pressure eliminated this source of instability.

A subtle but significant cause of periodic fluctuation in measurements is the level of liquid in the trap. Due to surface tension there may be a 10 mm. change in the level between the time at which drainage ceases and the time drainage again begins (Fig. 1A). Although the mechanism is not clear, this produces a concomitant change in flame luminescence. The installa-

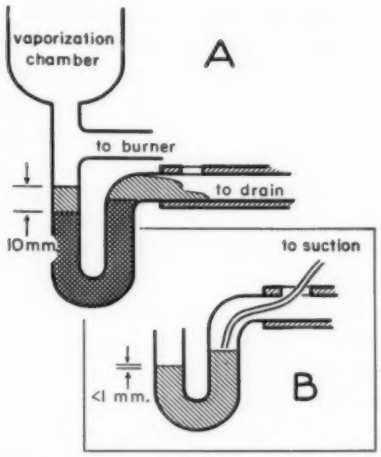

FIG. 1. Flame photometer drain trap. A. The liquid level rises from that shown by the cross hatch to that shown by the diagonal hatch before flow begins. The level then quickly drops to the initial level. B. Insertion of an aspirator tube lowers and stabilizes the liquid level. The level on the left is lower than the right due to the positive pressure in the vaporization chamber.

tion of an aspirator tip in the external limb of the trap reduced these level changes to less than 1 mm. (Fig. 1B).

Cleanliness of the nebulizer bore is essential for stable nebulization. Frequent passage of a wire through the bore together with periodic rinsing with a non-ionic detergent served to prevent accumulations of debris.

Measuring Circuit Stability. A minor light leak exists between the sodium-potassium filter disc and the housing. While insignificant under ordinary circumstances, at high sensitivity the leak permitted fluctuations in the ambient light to affect the sodium or potassium signal. A simple light-tight cover obviated this problem.

Another cause of occasional spurious readings was poor electrical connection in the operating switch contacts which tend to tarnish in a laboratory environment. The addition of another switch wafer permitted the paralleling of switch sections thereby reducing the likelihood of an occasional poor contact.

Optimum Common-mode Rejection. The approach described thus far has been to reduce to a minimum the fluctuations in the various factors which affect both the sodium (or potassium) ion signal and the lithium ion signal. This may be termed reducing common-mode variations. Another approach is to operate the photometer in such a fashion that the effect of such variations on the output is minimum; that is to say, that common-mode variations are rejected.

Since the relationship between the ion concentration at the flame and the electrical output of the photocell is non-linear, optimum common-mode rejection occurs when the sodium (or potassium) and lithium ion concentrations fall on similarly shaped portions of their respective concentration-output signal curves. Under these circumstances, changes in flame luminescence will produce proportional changes in the sodium (or potassium) and lithium signals and the final output will change only insofar as the two curves differ. Thus, for each sodium (or potassium) concentration there is an optimum lithium concentration for maximum common-mode rejection.

Such considerations are not significant when measurements are made at low sensitivity and over a reasonably narrow concentration range, but become important if one is to maintain stability when measurements are made at low concentrations and high sensitivity. At low concentrations of sodium (or potassium) the optimum lithium concentration will in general be preserved if the lithium concentration is proportionally reduced.

It should be pointed out that changes in electrical sensitivity by altering attenuation (or amplification) of the output signal do not alter the portion of the concentration-output signal being used. Such changes simply alter the output signal scale factor.

A simple alternative to changing lithium concentration for each range of sodium or potassium ion concentration is to change the amount of light reaching the lithium photocell. This may be achieved by using concentrations of lithium slightly greater than optimum and reducing the effective concentration by interposing fixed apertures or an iris diaphragm in the lithium light path. This technique is particularly useful in the case of muscle samples where the low sodium concentration requires a reduction in lithium concentration while the high potassium concentration would require an increase in lithium concentration for optimum stability and also optimal sensitivity. While this technique encompasses the non-linear properties of the photocell, it cannot compensate for the non-linear relationship between ion concentration and light emission due to self-absorption and interference.

TABLE I

Flame Photometer Dial Readings in 60 Serial Determinations Under Four Conditions on a Standard Containing Na 0.04 mEq./l. and Li 50 ppm.

Photometer	Mean	S.D.	S.E.M.	Coeff. of Variation
Unmodified & rinsed	747.1	44.2	5.7	±5.9%
Unmodified	752.0	36.4	4.7	±4.8%
Modified	773.6	14.6	1.9	±1.8%
Modified & aperture	827.3	10.4	1.4	±1.3%

Techniques for reducing the demand on the instrument for long term stability are well known, and will be mentioned only briefly. These include: allowing sufficient warmup for thermal equilibrium, reading unknown samples between closely spaced standards, checking the electrical zero between each sample. Such changes as the installation of a magnifying lens before the galvanometer screen and digital dial on the measuring potentiometer tend to reduce operator error.

RESULTS

Using a standard solution containing sodium 0.04 mEq./l. and lithium 50 ppm., sixty serial determinations were performed first without and then with a five-eighths inch aperture interposed in the lithium light path. All of the photometer modifications were then removed (but for the silicone coating on the funnel) and sixty further determinations were made. Following this, sixty determinations were performed in which the funnel was rinsed with ion free water between each sample. Table I summarizes the results of these determinations. The coefficient of variation was reduced from $\pm$ 5.7 per cent using the unmodified instrument with rinsing to $\pm$ 1.3 per cent with the fully modified instrument using the aperture.

SUMMARY

Stability is the major factor limiting increased flame photometer sensitivity. The following modifications were found to improve performance significantly:

1. Two stage gas and air pressure regulation.
2. Silicone coating the nebulizer.
3. Control of liquid level in the trap.
4. Occluding all light leaks.
5. Improving switch contacts.
6. Maintaining optimum common-mode rejection and sensitivity by a variable aperture in the lithium light path.

ACKNOWLEDGMENT

The author is indebted to Miss Zelda Wells for technical assistance.

STUDIES OF THE EPIDEMIOLOGY OF STAPHYLOCOCCAL INFECTION

II. Isolation of Staphylococci from the Milk of Nursing Mothers under Non-Epidemic Conditions[1]

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INTRODUCTION

Recognition of the importance of hospitals in the dissemination of staphylococcal disease has stimulated interest in the epidemiology and pathogenesis of these infections. Certain groups, notably diabetics, patients with malignant disease, newborn infants, and postpartum women, are particularly susceptible, but postoperative patients usually account for the greatest number of hospital staphylococcal infections (1). However, infections among newborn infants and their mothers often occur in epidemic form. Shaffer (2) has estimated that there were 500 nursery staphylococcal epidemics in the United States between 1954 and 1958.

One of the most frequently observed lesions during epidemics in nurseries is maternal breast abscess (3, 4, 5). Studies of these epidemics have indicated that the infant frequently becomes colonized with staphylococci before infection of the mother occurs, suggesting that the infant is important in the epidemiology of puerperal breast infection. It is likely that microorganisms are transmitted directly to the mother's breast from the oropharynx of the nursing infant.

Most epidemiological studies of infants and mothers have been done during outbreaks of staphylococcal infection in nurseries. The present study was undertaken to investigate the colonization of nursing infants and maternal milk with staphylococci over a prolonged non-epidemic period.

METHODS

The study was limited to patients living in Baltimore admitted to the obstetric ward of the Johns Hopkins Hospital. Nursing mothers available for follow-up study were included in this project.

Cultures of maternal milk were done daily in the hospital. After discharge, the pa-

[1] This work was supported by a contract with the Army Chemical Corps, Fort Detrick, Maryland and by Training Grant 2E-9, National Institute of Allergy and Infectious Disease, Bethesda, Maryland.

tients were visited weekly at home by a public health nurse who obtained the cultures and examined the patients for evidence of staphylococcal infection. Before milk cultures were obtained, the breasts were washed with phisohex® and rinsed with aqueous zephiran®. Following this, a jet of milk from each breast was expressed and caught in a sterile tube or Petri dish. The milk from each breast was cultured separately. Cultures of the mothers' and the infants' anterior nares and the infants' umbilical cords were also taken.

Specimens were streaked on blood agar plates and grown in trypticase soy broth for 18 hours at 37°C. Coagulase tests were done on all staphylococci isolated, using the method of Schaub (6). Coagulase-positive strains were bacteriophage typed by the method of Blair (7) with modifications (8) in current use in our laboratory. Typing was performed with routine test dilution strength bacteriophage. The set of bacteriophages used consisted of the following: Group I: 29, 52, 52A, 79, 80; Group II: 3A, 3B, 3C, 55, 71; Group III: 6, 7, 42E, 47, 53, 54, 75, 77, 83; Group IV: 42D, Miscellaneous; 81, 187, 44A.

RESULTS

Infant-mother Colonization

The investigation covered a fourteen month period. Forty infant-mother pairs were followed for three to ten weeks after delivery, with a median period of six weeks. Although coagulase-negative staphylococci were isolated frequently from the patients, the data to be presented are concerned only with coagulase-positive staphylococci. None of the patients developed overt staphylococcal disease during the study period.

Infant Colonization: In the hospital, coagulase-positive staphylococci were isolated from only two (5 per cent) of the forty infants. The average length of stay for these infants was two to three days. Twenty-six (65 per cent) were found to have coagulase-positive staphylococci on one or more of the home visits. Those infants who acquired coagulase-positive staphylococci at home usually did so soon after discharge, as shown in Table I.

Of the twenty-six infants from whom coagulase-positive staphylococci

TABLE I

Time When Coagulase-Positive Staphylococci Were First Isolated from the Infants' Nares and Cord and the Mothers' Milk

	No. of Infants who Became Positive for the First Time	No. of Mothers Whose Milk Became Positive for the First Time.
Hospital	2	1
Home		
1st visit	12	3
2nd visit	7	4
3rd visit	4	3
4th visit	1	1
5th visit	0	2
Total	26	14

TABLE II

Colonization of Mothers with Coagulase-Positive Staphylococci

Cultures	Hospital	Home
Milk and nose positive	0	9
Milk only positive	1	4
Nose only positive	11	15
Milk and nose negative	28	12

were isolated, in two the cord alone was colonized. Coagulase-positive staphylococci were found only in the nose in eleven cases and in both the umbilicus and the nose in thirteen cases. Culturing the umbilical cords, therefore, gave confirmatory evidence of colonization, but did not add appreciably to the number of carriers detected.

Maternal Colonization: The results of the cultures from the mothers are summarized in Table II. Coagulase-positive staphylococci were isolated from twenty-eight (70 per cent) of the mothers, twelve in the hospital and an additional sixteen at home. Ten of the eleven mothers with positive nose cultures in the hospital were found to have coagulase-positive staphylococci on the first day of hospitalization and may have been carriers prior to admission. A total of fourteen mothers (35 per cent) were found to have coagulase-positive staphylococci in their milk, one in the hospital and thirteen at home.

Bacteriophage Typing: No one bacteriophage type of staphylococci was encountered regularly during our investigation, reflecting the non-epidemic character of the study. Three infants and one milk sample were found to have bacteriophage type 80/81. Six milk samples had staphylococci that were typable but were other[2] than bacteriophage type 80/81, and seven milk samples had coagulase-positive staphylococci that were non-typable. These data are summarized in Table III.

In four cases the bacteriophage type found in the milk was isolated from the infant's nose or cord. Non-typable strains were found in both infant and milk in six cases, while in the remaining four the bacteriophage types found in the infant and milk were not the same.

Monthly incidence: An analysis of the monthly incidence of staphylococcal colonization reveals that coagulase-positive staphylococci were detected more frequently during the earlier months of the study (Fig. 1).

Sequence of Colonization: Comparison of the time of colonization of mother's milk and infant's nose or umbilical cord indicated that coagulase-positive staphylococci appeared sooner in the infant than in the mother's milk (Table I).

[2] Other bacteriophage types, each found on one occasion in mother's milk: VA4/77, 55/71, 3A/3B/3C/55, 53/54/VA4/75/77, 3A/44A,44A.

TABLE III

Bacteriophage Types of Staphylococci Cultured from Mothers' Milk and Infants' Nose or Umbilical Cord

Type Staphylococcus	Number of Infants	Number of Mothers
80/81	3	1
Typable strains other than 80/81	13	6
Non-typable	10	7
Coagulase-positive staphylococci not found	14	26

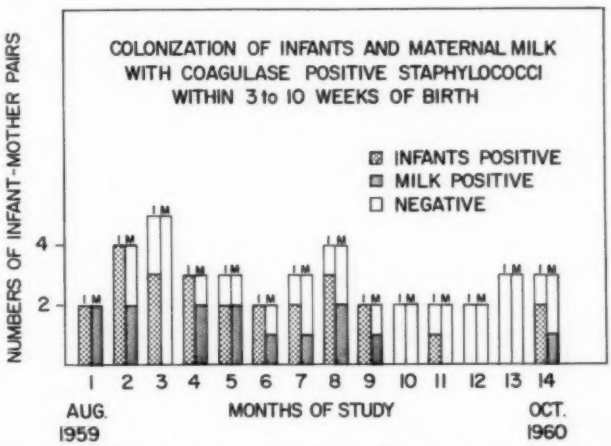

FIG. 1. The first half of each column represents the infant (I) and the other half the maternal milk (M). The total height of the column represents the number of infant-mother pairs *added* to the study during that month. The cross-hatched areas represent infants with coagulase-positive staphylococci, and the stippled areas represent mothers with coagulase-positive staphylococci in their milk. The period of time each infant-mother pair was followed after birth ranged from 3 to 10 weeks. This figure indicates the changing colonization rate observed during the study.

Quantitative Milk Counts

To define further the process of staphylococcal colonization of mother's milk, quantitative counts of breast milk were started in October 1960. Quantitative counts were done by adding one milliliter of milk to 9 milliliters of broth. The broth was then refrigerated in a portable ice chest until brought to the laboratory where agar pour plates were made from serial ten-fold dilutions. Colony counts were then determined after incubation at 37°C. for eighteen hours.

Quantitative counts of sixty-eight samples of breast milk are shown in Figure 2. Fifty-nine per cent of milk samples had counts above 1,000 organisms per milliliter, and 31 per cent had counts above 10,000 organisms per milliliter. The highest count was 1,256,000.

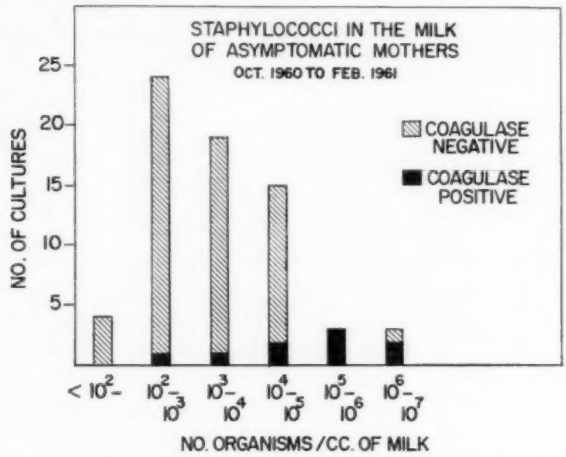

FIG. 2. This figure indicates the numbers of staphylococci found in 68 samples of breast milk as determined by quantitative bacterial counts. It can be seen that nearly a third of these samples had counts greater than 10,000 organisms per milliliter.

As can be seen in Figure 2, most of the milk cultures done during this period revealed coagulase-negative rather than coagulase-positive staphylococci. The bacterial counts done on the milk samples containing coagulase-positive staphylococci tended to be higher than counts done on milk containing coagulase-negative staphylococci. The number of observations involved, however, was too small to permit any conclusions. Quantitative counts of maternal milk are being continued.

It was anticipated that we might have an opportunity to study the bacterial counts in the milk of mothers with clinical mastitis, but up to this time such an opportunity has not arisen.

DISCUSSION

The incidence of maternal breast abscess is extremely high in nursery epidemics of staphylococcal disease. Ribble and Eichenwald (9) have recently studied such an epidemic in which thirteen of thirty-nine nursing mothers developed clinical mastitis, an incidence of 33 per cent. The importance of the infant as a reservoir for staphylococci was emphasized by the finding that not one of thirty mothers of formula fed babies studied at the same time developed mastitis.

Studies of other epidemics of staphylococcal infection also have indicated infant to mother transmission. In an outbreak reported by Ravenholt (5), the median time of onset of infant pyoderma was seven days after delivery, while the median time of onset of maternal mastitis was eleven days. In

twelve cases of mastitis studied by Monro and Markam (10), the type of staphylococcus found in the breast abscess could be isolated from the infant in eleven cases, but from the mother's nose in only three cases. Our data support the concept that the infant serves as a source of staphylococci invading the breast.

The mechanisms by which staphylococci are transmitted from the infant to the breast have been investigated by Wysham (4). He cultured the milk remaining in bottles after the feeding of formula to infants. Staphylococci could usually be found in the bottles used by colonized infants, but not in the bottles used by control, uncolonized infants. Wysham postulated that the negative pressure in the bottle carried organisms through the pores of the nipple, and he suggested that a similar mechanism might be operative during breast feeding. He also noted that fissures or other defects of the nipple need not be present for staphylococci to invade the breast.

Colonization of the breast with staphylococci has also been shown to exist in the absence of manifest disease (4, 11, 12, 13). Duncan and Walker (11) in 1942 found staphylococci in the milk of more than 90 per cent of nursing mothers. Although quantitative counts were not done, bacteria were seen by direct microscopic examination of fresh milk in a small number of cases. In addition, staphylococcal mastitis in cows, which has long been a significant veterinary problem, has also been shown to exist in an asymptomatic form (14, 15).

The absence of clinical mastitis among mothers in our study is not surprising in view of the comparatively low incidence of this disease under non-epidemic conditions. Dipple and Johnson (16) in a review of 20,258 deliveries at the Johns Hopkins Hospital over a thirty-eight year period found only 113 cases of puerperal mastitis, an incidence of 0.56 per cent. However, our data indicate that colonization of the breast with significant numbers of staphylococci occurs frequently. Although infant colonization with coagulase-positive staphylococci is also common, pyoderma under non-epidemic conditions usually occurs in less than 1 per cent of infants (17). The explanation for these low attack rates lies in the relationship between the virulence and number of staphylococci inoculated and the susceptibility of the host.

The most commonly used index of the virulence of staphylococci is their ability to produce coagulase. Coagulase-positive staphylococci are responsible for most staphylococcal infections, while coagulase-negative staphylococci are rarely implicated as the cause of disease. The exact role that coagulase plays in the pathogenesis of staphylococcal infection, however, has not been precisely defined (18, 19).

With the development of bacteriophage typing, it has become apparent that strains of coagulase-positive staphylococci differ in the frequency with

which they cause infection. The 80/81 strain in particular has been a frequent cause of nursery staphylococcal epidemics. Yet in animal experiments this strain has not been found to be more capable of initiating staphylococcal infection than non-epidemic strains. Thus, Bennett, Minchew, and Fekety (20) introduced the term "epidemiological virulence" to describe the dissociation between the laboratory and clinical behavior of this strain.

Initiation of infection with staphylococci is related to the size of inoculum. Elek (21) has shown that at least 1,000,000 staphylococci must be injected intradermally to produce abscesses in human volunteers. The number of bacteria inoculated into the breast may similarly be related to the development of clinical mastitis.

A relationship between number of organisms and the presence of disease has been established in the case of urinary tract infections. Kass (22) has shown that clinical pyelonephritis is most often found in patients whose urine contains more than 100,000 organisms per ml. We are now investigating the possibility that an analogous relationship exists between quantitative bacterial counts in milk and the occurrence of clinical breast infection.

Host factors, such as anatomical variations in the breast, degree of engorgement, and technic of feeding may also be important in the pathogenesis of mastitis.

The high incidence of maternal mastitis in nursery epidemics and the ease with which staphylococci may be transmitted from infant to mother suggest that the occurrence of mastitis is an indication for weaning. Continued nursing in this situation could result in repeated inoculation of virulent staphylococci into the mother's breasts and cause extention of the infection. The recommendation, in a report of the Yale Rooming-in Project (23), that mothers with unilateral mastitis continue to nurse on the unaffected side seems unwise in light of present knowledge.

Handelman (24) has recently recommended temporary cessation of breast feeding in mothers with mastitis who are reluctant to discontinue nursing. She suggests pumping the breasts during the period of infection and resumption of nursing when the infection subsides. This plan could lead to reinfection of the mother, however, if the infant were still a carrier of the pathogenic organism when nursing was resumed.

Our finding of significant staphylococcal colonization of breast milk during a non-epidemic period might be applied to management of breast-feeding during epidemics. During nursery epidemics, significant maternal morbidity in the form of breast abscess might be avoided by the interdiction of breast feeding by mothers whose infants are colonized by the epidemic strain.

Reports in the literature differ in point of view concerning the way

staphylococci are spread in the newborn nursery. Some authors emphasize the role of the nurse carrier (3, 25, 26). Others (27, 28, 29) stress the infant as a source of staphylococci: and still others stress the environment as the reservoir (30, 31, 32). These divergent views reflect the complexity of the situation in which several of these factors may be operative and which may differ from one nursery to the next (33).

In our study, coagulase-positive staphylococci were recovered from only 5 per cent of the infants in the hospital nursery. The low rate of colonization was not due to the absence of carriers among nursery workers. During the time covered by the study, the carrier rate for coagulase-positive staphylococci among nurses in this hospital was approximately 50 per cent. At the present time, cultures of all nursery personnel are taken monthly. Carriers of the 80/81 strain are now removed from the nursery, although this policy was not in effect when our study was carried out. These individuals are treated with an intranasal antibiotic ointment for a ten day period. If the 80/81 staphylococci carrier state persists, the individual is transferred to a different section of the hospital. This policy reflects our feeling that the newborn infant is particularly susceptible to infections caused by bacteriophage type 80/81 staphylococci.

This study supplements a previous report dealing with staphylococcal colonization of pediatric outpatients at this hospital (34). Colonization with coagulase-positive staphylococci was found to occur in 24 per cent of infants under five months of age, but in only 6.6 per cent of infants five to twelve months of age. That 65 per cent of infants in our study were colonized with coagulase-positive staphylococci in the neonatal period further suggests a diminishing incidence of carriers during the first year of life.

There was a fluctuation in the colonization rate of infants and mothers during the course of the present investigation. This fluctuation during a non-epidemic period points out the importance of collecting data over a prolonged period of time before conclusions can be drawn concerning the colonization rate.

SUMMARY AND CONCLUSIONS

Asymptomatic infection of the nursing mother's breast with staphylococci was found over a prolonged non-epidemic period.

Quantitative bacterial counts suggested that as many as one million staphylococci per ml. may be present in breast milk in the absence of clinically apparent disease.

Although during nursery outbreaks the incidence of mastitis due to a single epidemic strain is high, no cases of breast abscess occurred in our patients whose milk was colonized with a variety of strains of coagulase-positive staphylococci.

Our data support the concept that staphylococci are transmitted to the breast from the oropharynx of the nursing infant. The implications of this concept in regard to the management of breast feeding are discussed.

Five per cent of infants were colonized with coagulase-positive staphylococci during their relatively short hospital stay. The incidence of colonization at home was 65 per cent, although the rate varied during the fourteen month study period.

Although infants as a group appear to be especially prone to colonization with coagulase-positive staphylococci, the route by which they acquire these organisms is uncertain.

ACKNOWLEDGMENTS

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HEPATIC COMA AS A COMPLICATION OF PARA-AMINO SALICYLIC ACID THERAPY[1]

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The use of para-amino salicylic acid (PAS), alone or in conjunction with other drugs, in the treatment of tuberculosis is wide-spread, efficacious and accompanied by relatively few serious side reactions. Disturbed liver function, with and without jaundice, has been reported as a complication of PAS therapy. This type of untoward reaction has usually developed in those patients in whom early indications of hypersensitivity such as fever and/or rash, were overlooked or ignored, or in whom there was inadvertent re-administration of the drug after such toxic signs were noted. Death from such hepatic damage has been reported (1).

The onset of hepatic coma is usually accompanied by a rise in blood ammonia concentration. Methods aimed at lowering blood ammonia levels are employed in the treatment of hepatic coma. Most disagreement in the literature centers about the efficacy of these methods in the treatment of so-called "endogenous" coma (2 to 7). The present patient is presented as a case of hepatic decompensation, i.e. "endogenous coma", precipitated by the use of PAS and successfully treated with what we consider standard methods of treatment for ammoniagenic coma.

Case Report: C.M., 24 yr. old n.f., B.C.H. #300715.

The patient was admitted on 7–19–60 with a dense infiltrate in the left upper lobe and sputum positive for acid-fast bacilli. She was a known epileptic since age thirteen months and had been controlled with dilantin and phenobarbital. The patient was asymptomatic on admission, the disease having been discovered on routine chest x-ray survey. Physical examination was within normal limits. Laboratory studies on admission were within normal limits. The hematocrit was 37 mm. the white blood cell count 3,800 and the differential as follows: non-segmented forms 2, polymorphonuclear leukocytes 48, eosinophiles 2, basophiles 4, lympocytes 36 and monocytes 8 per cent. The fasting serum sugar was 95 mg. per cent and the serum urea nitrogen was 10 mg. per cent. She was continued on phenobarbital 30 mg. t.i.d. and dilantin 100 mg. t.i.d. and started on standard doses of PAS and INH on 7–20–60. She was afebrile until August 18. At this time she began to manifest a gradual increase in temperature (See Fig. 1). On August 26 her temperature was 101°F. and a skin rash was noted. The first liver function tests (See Table I) were drawn on this day and the PAS and INH were discontinued after the morning dose had been given. On September 7, 1960 the patient complained of nausea and vomiting, but she was afebrile. The following day slight icterus was noted for the first time. On physical examination no masses or organs were felt but she was

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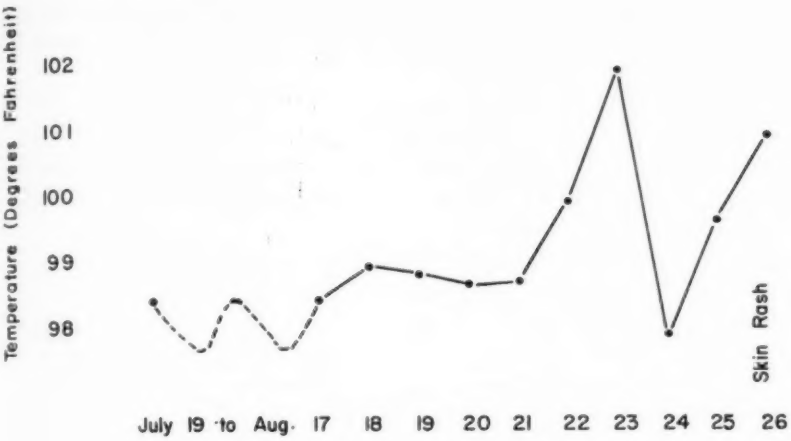

FIG. 1. Temperature course prior to skin rash.

sensitive in the right upper quadrant. On September 13 she became drowsy in the morning, icterus deepened, vomiting ensued and her condition deteriorated. She became less responsive and was stuporous by the late afternoon. The liver was still not palpable and liver dullness extended only to one to two centimeters above the right costal margin. INH was reinstituted (300 mg. t.i.d.) with prednisilone added (20 mg. q.8 h.). On September 14 she became semi-comatose. Ten per cent I.V. glucose with added B and C vitamins was started as well as menadione 5 mg. I.M. Tube feeding of a low fat, low protein, high carbohydrate mixture was instituted. Neomycin 2 gm. q.6 h. was given through the stomach tube. The patient was transferred to the acute medical ward on this day. At the time of transfer the tube feeding mixture was changed to contain carbohydrate only. She was continued on neomycin, vitamins K, B & C as outlined above, and 100 mg. cortisone I.V. q.8 h. was started. On September 15 deep coma persisted. A total of 50 gms. of sodium glutamate was given intravenously this day. An EEG was done on September 16 which showed slow, low voltage waves compatible with hepatic coma. Another 25 gms. of sodium glutamate was given intravenously. By September 17 she responded to noxious stimuli. On September 19 she asked for water and, the next day, for food. She was given a diet containing 20 gm. protein at this time. Gradual reduction in steroids was started. On September 22 her protein intake was increased to 40 gm. per day. Her affect was noted to still be flat. Diarrhea developed on September 26 and the neomycin was discontinued. By September 28 she was alert and her diet was increased to contain 50 gm. protein. On October 4 she was placed on a regular diet. She discontinued all medications except streptomycin, INH and menadione on October 6. On October 7 she had a grand mal seizure and was placed back on phenobarbital 30 mg. t.i.d. She was transferred back to the tuberculosis hospital on October 18 as an ambulatory patient. On November 12, 1960, two months after the onset of coma, a liver biopsy was done. The report of the liver biopsy was as follows: The architecture of the liver is altered by focal fibrosis in occasional portal areas with distortion of the limiting membrane of the lobules, occasional foci in lobules replaced by collapsed reticulum containing some large mononuclear cells. In one area the septal fibrosis of portal areas

extends to and connects with central veins. A few foci of large ballooned regenerating liver cells are noted. Impression—Focal fibrosis of liver, portal and septal, compatible with healed phase "toxic hepatitis".

TABLE I

Liver Function Studies

	SUN[1]	Transaminases		T.T.[4]	C.F.[5]	Alk.[6] P-ase	Bili.[7] T-D		Proth.[8]	A/G[9]
		G-O[2]	G-P[3]							
8-25-60		57	49	5.0	1+		<1.0	<0.6		
9-9-60		>4000	2840	20		44.6	12.5	4.9		3.3/5.0
9-13-60		1380	—	25			30.0	14.4	<10%	3.6/5.4
9-14-60				23						
9-15-60	12	1080	1410			29.2	29.6	22.4	11%	
9-16-60		1320	1890			27.1	19.2	10.8	13%	
9-19-60	9	300	700							
9-20-60		400	1060				15.4	9.2	23%	
9-23-60		170	440			17.5	16.4	9.0		3.2/4.2
9-26-60		59	560			28	12	6.8	40%	
10-3-60		59	73			29.9	7.2	4.3	68%	
10-26-60		38	33	11.3	1+		2.1	1.0		
1-9-61		24	32	6.5	1+		<1.0	0.6	100%	

Electrolytes remained within normal limits (Na, K, Cl, CO_2)

1. Sun—serum urea nitrogen, 2. G-O—glutamic-oxalacetic, 3. G-P—glutamic-pyruvic, 4. T.T.—thymol turbidity, 5. C.F.—cephalin flocculation, 6. Alk. P-ase—alkaline phosphatase, King-Armstrong units, 7. Bil. T.D.—bilirubin, total, direct, 8. Proth.—prothrombin time, 9. A/G—albumin and globulin levels.

DISCUSSION

When the first blood ammonia determinations were done (See Table II), the patient was in profound coma. After it was discovered that the ammonia levels were markedly elevated and in a range consistent with hepatic coma, measures designed to reduce the ammonia levels were instituted. As can be noted in the protocol these included a protein free diet, oral neomycin (to reduce bacterial flora in the gut), enemas (to remove bacteria and nitrogenous material from the lower bowel), and intravenous sodium glutamate. Sodium glutamate aids in reducing blood ammonia levels by combining with ammonia in the msucle to form glutamine, a non-toxic product (9).

The above measures were instituted in order to lower the blood level of ammonia. We believe that elevated ammonia levels are an important factor in the pathogenesis of hepatic coma. A theory has been proposed and substantiated by laboratory data (10), that ammonia causes coma by reducing available substrate for the brain by depleting the Krebs cycle intermediates. There are undoubtedly other factors operating in hepatic de-

TABLE II

Blood Ammonia Levels

Date	State of Consciousness	Ammonia-N (γ/cc)*		Intravenous Glutamate Treatment (See text for other treatment)
		Art.	Ven.	
9-14-60	Deep coma, no response	3.94	3.66	
9-15-60	9 A.M. Deep coma, no response	2.70	2.56	Approx. 37½ gms. received, running in at time of determination.
	3 P.M. Comatose, response to pain only		2.88	50 gms. total received. I.V. disc. 30 minutes before determination.
9-16-60				25 gms.
9-20-60	Awake but slightly confused			
9-26-60	No impairment		1.38	
10-28-60	No impairment		1.13	

* (Normals in our laboratory[8] 0.6–1.2γ/cc).

compensation, but if the coma produced by elevated ammonia levels persists, this in itself can ultimately be lethal because of subsequent decrease in overall cerebral metabolism. Therefore it is imperative to treat this parameter in hopes that the underlying liver disease may be reversible. It has been stated in the literature that sodium glutamate and other measures designed to reduce blood ammonia levels are of little use in "endogenous" hepatic coma, i.e. primary hepatic decompensation, as opposed to their usefulness in "exogenous" coma, i.e. coma precipitated by gastrointestinal bleeding, administration of ammonium salts, etc. (2 to 5). This is true only if one accepts the idea that there is no chance for a reversal of the underlying disease process. The complete recovery of our patient is a contradiction of such philosophy. It is believed that this is the first reported case of a patient who developed hepatic coma from PAS toxicity who survived.

The literature contains several excellent reviews of the subject of PAS toxicity (1, 11, 12). As has been pointed out by Simpson and Walker, hepatic injury is likely to occur only if the early warning signals of PAS toxicity, such as fever, rash and joint pains, are ignored. The more severe manifestations are apparently related to delay in stopping the drug. The patient under discussion, after a month of being completely afebrile, manifested her first sign of toxicity as fever. After eight days of fever she developed a rash, and only at this time was the drug discontinued.

In the large series reviewed by Simpson and Walker the onset of toxic symptoms was noted in over half of the cases between the beginning of the third and the end of the fifth week of PAS administration. They also point

out that a gradual rise in temperature is common although this finding had not been previously commented upon in the literature. Fever was noted as one of the early signs of toxicity in over ninety-eight per cent of their cases, being absent in only five of two hundred and twenty-nine cases. In both of these aspects the patient under discussion is "typical".

CONCLUSION

A case of hepatic failure with ammonia intoxication, precipitated by PAS toxicity, was successfully treated by measures including those designed to lower blood ammonia levels. The potential toxicity of PAS, with the premonitary sign of rising temperature, is re-emphasized as well as the fact that drug induced hepatic coma can have a favorable outcome.

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LABORATORY-ACQUIRED TYPHOID FEVER

Infection with a Laboratory Strain of *Salmonella typhosa* Isolated Forty-one Years Earlier.[1]

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Salmonella typhosa is a relatively common cause of laboratory-acquired bacterial infection (1 to 3). In a survey of laboratories in the United States in 1951 Sulkin and Pike (1) obtained data on fifty-eight cases of typhoid fever among a total of 775 bacterial infections acquired as a result of laboratory work. Infection is usually caused by a recently isolated strain of *S. typhosa* or organisms present in stool or urine, but may be related to "laboratory" strains that have been subcultured on artificial media for as long as six years (2 to 4). Infection usually occurs as the result of an accident such as aspiration of a bacterial suspension through a pipette or splattering of infectious material, although occasionally no known break in technique has occurred. Immunization with typhoid vaccine does not confer complete protection against laboratory-acquired infection with *S. typhosa*, but the disease may be less severe in immunized than in non-immunized individuals (3, 5).

The present report describes a case of typhoid fever in a medical student who was engaged in a research project utilizing *S. typhosa*. This case is of unusual interest because the organism apparently responsible for the infection is a well-known "laboratory" strain (Ty2) of *S. typhosa* that was originally isolated forty-one years prior to the present episode.

CASE REPORT

C.O., a twenty-four year old male medical student, began a study of factors influencing clearance of the Ty2 strain of *S. typhosa* from the blood stream of the rabbit on June 12, 1959. He had received 1 ml. of typhoid vaccine two years prior to beginning the series of experiments.

In the experiments performed by the student infectious inocula containing approximately 10^9 viable bacilli per ml. were prepared by harvesting the growth on agar slants with saline solution. Appropriate dilutions of bacterial suspensions were inoculated intravenously into rabbits, and blood specimens were collected at intervals of time for enumeration of bacteria by conventional colony-counting techniques. No known labo-

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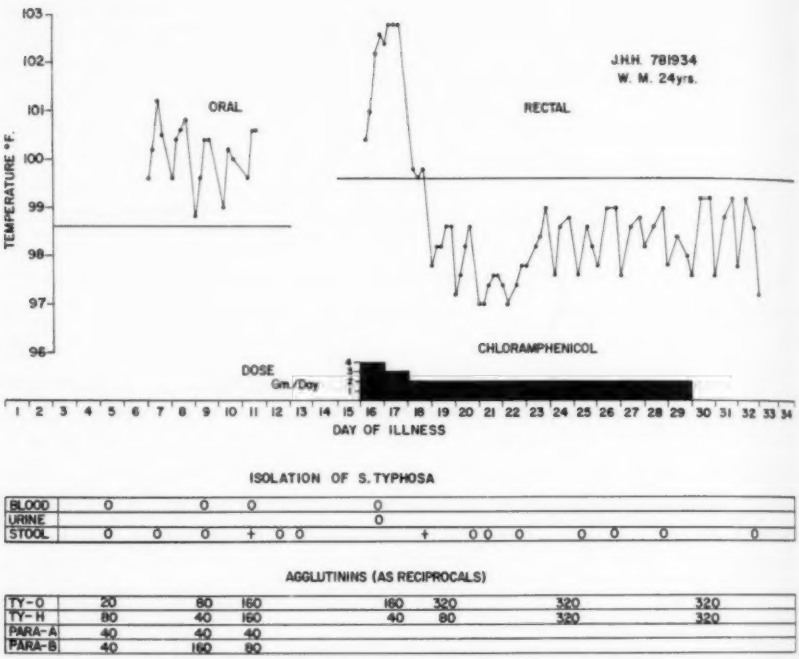

FIG. 1. Course of illness in patient with laboratory-acquired typhoid fever

ratory accidents had occurred and the student was not aware of aspirating infectious material while pipetting. The student had had no known contact with strains of *S. typhosa* other than Ty2. The mouthpieces of all pipettes were plugged with cotton and the usual aseptic techniques were employed.

The student was well until July 26, 1959, when he noted the abrupt onset of fever and headache. The course of illness is outlined in Figure 1. The most prominent symptoms during the first week of illness were anorexia, mild constipation, and headache. He was afebrile during the morning hours but developed fever with temperatures ranging from 100 to 101.5°F. during the afternoon and evening. A prompt decrease in temperature and profuse diaphoresis occurred after administration of 0.3 gm. of aspirin. On the fourth day of illness a non-tender spleen was palpated 3 to 4 cm. below the left costal margin, and the liver edge was palpated 3 to 4 cm. below the right costal margin.

The patient remained at bed rest during the second week of illness with little change in symptoms. Fever, hepatomegaly and splenomegaly persisted, and on the ninth day of illness, a rectangular, erythematous macule was observed in the skin over the left lower quadrant of the abdomen. The cutaneous lesion did not blanch on pressure and disappeared completely after two days.

During the first two weeks of illness blood leukocyte counts ranged from 4700 to 5000 cells per cubic millimeter, and differential counts showed 18 to 19 per cent juvenile forms, 28 to 38 per cent polymorphonuclear leukocytes, 26 to 30 per cent lymphocytes, 3.5 to 4 per cent atypical lymphocytes, and 17 to 20 per cent monocytes. The

bilirubin was less than 0.8 mg. per 100 ml., cephalin flocculation was negative, thymol turbidity was 2.1 units, alkaline phosphatase was 4.1 Bodansky units, and the total serum proteins were 7.7 gm. per 100 ml. (albumin 4.4 gm., and globulin 3.3 gm.).

Four blood cultures taken during the first sixteen days of illness and three specimens of stool collected on the fifth, seventh and ninth days of illness were negative for *Salmonella*. However, *S. typhosa* was isolated from a stool specimen collected on the eleventh day. The patient was hospitalized and therapy with chloramphenicol was initiated on the fifteenth day after onset of symptoms. After three days he was afebrile, and began to take added interest in his surroundings and eat voraciously. *S. typhosa* was again isolated from stool two days after institution of therapy with chloramphenicol but subsequent cultures were negative for *Salmonella*. The spleen could not be palpated after the seventh day of therapy. Chloramphenicol was continued for a total of fourteen days, and the patient was discharged from the hospital on the thirty-third day after onset of symptoms. As shown in Figure 1, periodic examination of serum during the course of illness revealed a significant increase in antibody titer against typhoid O and H antigens. The patient has shown no evidence of clinical or bacteriologic relapse during a follow-up period of eighteen months.

Although the circumstances suggested strongly that this infection was acquired in the laboratory, additional evidence was sought by comparing certain biological characteristics of the organism isolated from the patient with those of the Ty2 strain. It was shown that the two strains were of the same bacteriophage type (E_1) and gave identical biochemical reactions in Kligler's iron agar. Colonial appearance on meat extract agar was the same when examined by the oblique-lighting technique (6). Serological examination revealed the presence of Vi, O, and H antigens in both cultures. In the viable state, they were highly motile (contained factor d flagellar component), each agglutinated to a titer of 1:320 in a Vi antiserum, and agglutinated to titers of 1:40 and 1:80 in an O antiserum. The relative O-inagglutinability of these organisms was eliminated when killed cells (boiled for one hour) were employed, inasmuch as both cultures then agglutinated in the O antiserum to a titer of 1:1280. Absorption studies, employing a Vi antiserum of known titer showed that the two strains contained equal quantities of Vi antigen. The mouse virulence of the isolate from the patient was essentially the same as that of the Ty2 strain. Employing saline suspensions of the organisms for intraperitoneal inoculation of BALB/c mice, an LD_{50} of 3.09×10^6 bacilli was obtained for the strain isolated from the patient, as compared to an LD_{50} of 3.01×10^6 for Ty2, while intracerebral LD_{50}s for the same strain of mice were 2850 and 2070 cells respectively. The similarity of biological characteristics of the strain of *S. typhosa* isolated from the patient and the Ty2 strain with which the patient had been working adds strong support to the concept that this was a laboratory-acquired infection caused by *S. typhosa* Ty2.

The Ty2 strain used by the patient was obtained from Dr. Maurice Landy of the National Institutes of Health, Bethesda, Maryland, and had been subcultured repeatedly on trypticase-soy agar in this laboratory for two years prior to the present infection.

DISCUSSION

The Ty2 strain of *Salmonella typhosa* which apparently caused the present case of laboratory-acquired typhoid fever was originally isolated from a fatal case that occurred during a typhoid outbreak in Cherson, Russia in 1918 (7). This strain has shown unusual stability in maintaining anti-

genic composition and virulence for animals despite subculture on plain nutrient agar for periods of many years (7), or continuous culture for 1 to 3 weeks (8), and because of its stability has been widely used in experimental work and in the preparation of typhoid vaccine (7). The precise passage history of the strain is unknown; however, it is of interest that despite residence outside the human host for forty-one years, and hundreds, perhaps thousands of subcultures on artificial media, the organism is still capable of producing typhoid fever in man. The recent studies of Edsall and coworkers (9) have shown that the Ty2 strain is also pathogenic for chimpanzees infected by the oral route, and that the disease in these animals has many of the characteristics of typhoid fever, including intestinal infection, fever, bacteremia, and typical histological changes.

The dose of *S. typhosa* required to produce typhoid fever in man is unknown. Although the infectious dose is almost certainly influenced by factors such as virulence of the strain and resistance of the host, it is generally assumed to be closer to several thousand rather than billions of organisms (9). The patient described in this report worked with bacterial suspensions containing no more than 10^9 viable units per milliliter, and experienced no apparent laboratory accidents associated with gross contamination of the mouth or face with infectious material. These observations suggest that the number of bacilli initiating the present infection was considerably less than 10^9 viable units.

It is well known that immunization with vaccine or recovery from disease does not provide complete protection against subsequent infection with *S. typhosa* (5, 10 to 13). However, there is evidence that the course of disease is less severe in immunized than in non-immunized individuals (1, 3, 5). In the 168 cases of laboratory-associated typhoid fever described by Schafer (3), the mortality rate was 3 per cent in sixty-six previously immunized persons in contrast to a mortality of 13 per cent in ninety-two non-immunized subjects. The patient described in this report had received partial immunization against typhoid, and whether this was related to the relatively benign course of his disease is unknown.

SUMMARY

A case of laboratory-acquired typhoid fever apparently caused by the Ty2 strain of *Salmonella typhosa* is described. This strain was isolated forty-one years prior to the present episode and has been subcultured hundreds, perhaps thousands of times on artificial media.

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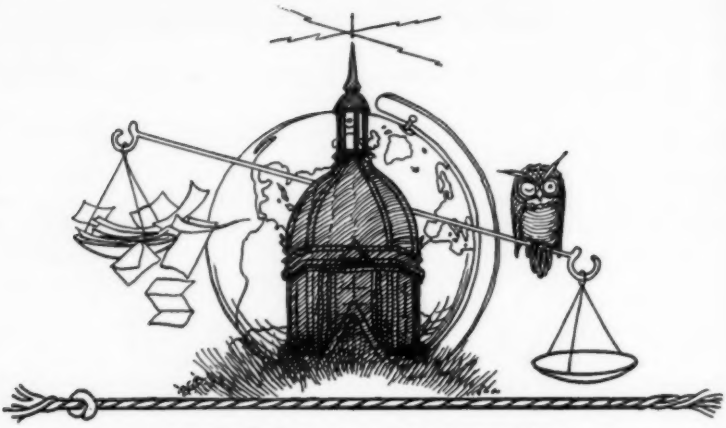

BOOK REVIEWS

Essential Hypertension. An International Symposium. Sponsored by Ciba-Basle. Edited by K. D. Bock and P. T. Cottier. 392 pp., $9.00. Springer-Verlag, Heidelberg, West Germany, 1960. Available at Intercontinental Medical Book Corp., New York, N. Y.

In June 1960, a symposium was held in Berne, Switzerland, sponsored by Ciba. The publication of the symposium contains both prepared communications by some of the best known international figures in the field of hypertension, such as Page, Pickering, Platt, Schroeder, Hood, Grollman, Wilson, Freis, and Dustan, and verbatim reports of interesting and vigorous discussions.

In a field in which there is no lack of speculation, Dr. Cottier's chapter on renal hemodynamics seems difficult to follow. Charts of the mean clearances of different groups of patients using different drugs and comparing this with mean arterial pressures appear to give the impression that hydralazine has something particular to offer in the treatment of hypertension with renal disease. This supposition is not born out by his evidence which is conflicting and selects features so as to give the impression already referred to.

Dr. Plummer's chapter on page 240, in addition to providing some interesting pharmacologic data, suffers from lack of balance. There is no good evidence that syrosingopine "constitutes a further important advance in antihypertensive therapy." It is of interest that Hilden and others have found that tolerance develops fairly often with guanethidine, contrary to some other reports in the world's literature.

Dr. Reubi's chapter on the late effects of hypotensive therapy is of interest as one of the few attempts to assess the value of such treatment in mild and moderate hypertension. His findings suggest that treatment does not delay renal deterioration in hypertension associated with retinal changes Grades I and II, while in Grades III and IV, reports strongly suggest that in very severe and malignant hypertension treatment is of value in arresting renal deterioration.

These findings are of particular interest in view of the almost universally poor response experienced in our own clinics if we attempt to seriously lower the blood pressure in severe or malignant hypertension. This chapter and other items in the symposium suggest that the type of hypertensive disease with inexorably progressive renal failure is something which is unusually common in our colored population and is not

as frequently reported, either amongst white patients elsewhere in the United States or in European hypertensive clinics. Care must be taken to describe carefully the racial and other background of hypertensive populations undergoing studies and in not generalizing the results from one clinic to all hypertensives. The speakers or discussers of the symposium have not stressed this difficulty.

This, however, should not detract from a well worthwhile discussion, which should be read critically and which seems a valuable up-to-date addition to the literature on hypertension.

FREDERICK WOLFF

Haematology and Blood Groups. Edited by D. A. G. GALTON and K. L. G. GOLDSMITH. 169 pp., $4.00. The University of Chicago Press, Chicago, Ill., 1961.

This volume is reprinted from the British Medical Bulletin, *15:* Nos. 1 and 2, 1959. The first section deals with a series of papers concerned with the red blood cell. Factors necessary for growth development of the red cell are reviewed in specific papers on iron, vitamin B_{12}, and folic acid. The biosynthesis of hemoglobin and the chemistry of abnormal hemoglobins, are also reviewed. Several papers deal with the destruction of red cells, and there are three brief papers concerning lymphocytes and leukemia.

The second half of the volume consists of a group of papers which review the blood groups from the point of view of genetics, clinical importance, biochemistry and anthropology. The entire volume is an important contribution. The authors selected to review these subjects are all actively engaged in the investigation of the problems relating to their subjects. The articles are well referenced, and the illustrative material is clearly presented. The total effort has produced a volume which should be of value to anyone interested in hematology.

JULIUS R. KREVANS

Pharmacology. The Nature, Action and Use of Drugs, 2nd edition. By HARRY BECKMAN. 805 pp., $15.50. W. B. Saunders Co., Philadelphia, Pa., 1961.

The appearance of the second edition of Dr. Harry Beckman's Pharmacology underlines the difference between multi and single author textbooks. Dr. Beckman has taken full advantage of the single author method. The style is even and consistent. The book reads easily and the sections are generally speaking well balanced although in future editions it will be necessary to expand the chapter on the nature of drug action.

For a general textbook of this type the single author is always faced with the problem of writing about subjects with which he has no first hand experience. The rapid expansion of pharmacology makes this a particularly difficult subject. Dr. Beckman has read very widely of the literature and has done as much as any single man can to sift and synthesize the relevant facts.

Occasional sections, however, lack the authority of someone who is familiar with the field.

In summary for those who want a clear and readable exposition of pharmacology without too much detail and without too much critical discussion of fine points Dr. Beckman has much to offer.

JOHN R. TROUNCE

Medical-Surgical Nursing, 2nd edition. By KATHLEEN SHAFER, JANET SAWYER, AUDREY MCCLUSKEY and EDNA BECK. 876 pp., $8.75. The C. V. Mosby Co., St. Louis, Mo., 1961.

This is one of the few texts, perhaps it is the only one, which attempts to combine medical and surgical nursing. In this second edition a number of changes have been made, although the book retains the qualities that recommended the earlier edition, and the fundamental plan remains essentially unchanged. New material, some more comprehensive discussions, and some rearrangement of content greatly enhance its value. An important feature is the authors' concern with not only the physical aspects of patient care, but also with the need for nurses to give thought to the social and emotional factors.

This edition, as the one preceding it, is divided into two main parts: 1) General considerations, an increase in chronic illness, need for rehabilitation, etc., and 2) Nursing related to specific medical and surgical care. The content is arranged under headings and subheadings which highlight the organization of each chapter. The combined subject area covered by the book is so broad that, though the general treatment is excellent, it is probable that for depth in the specialties, such as ophthalmology, additional references or periodical readings would be required.

The new volume is attractive in format; it is set up with two columns to the page, a style which lends itself to easy reading. The text is augmented by many excellent photographs and illustrations. Direct, practical questions at the beginning of each chapter draw for their answers on previous learning, and arouse interest in the content. There is an up-to-date bibliography at the end of each chapter, with an indication of those references particularly suitable for student reading. A well-arranged table of contents and index contribute to making the book convenient as a text and add to its usefulness as a reference.

As all of the material in the book is focused on the practice of nursing, rather than on the study of disease entities as such, it is, in my opinion, one of the best textbooks offered for basic instruction in medical and surgical nursing; it should prove equally valuable as a reference for the student in her later years in the nursing program.

MARGARET E. COURTNEY

Clinical Bacteriology. By E. JOAN STOKES. Foreword by A. A. Miles. 309 pp., $7.00. Edward Arnold, London, 1960. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

This book describes the approach to the laboratory investigation of infectious problems that is used in an eminently successful British clinical laboratory. The methods are rigorously scientific; yet practical aspects are emphasized. The style is entertaining, and even the casual reader will find the book abounds with tricks of the trade and sage observations born of long experience. Less happily, the author has attempted to cover a vast amount of material briefly, and the book suffers from being neither a definitive treatise nor a detailed manual. The chapters on antibacterial drugs, clinical immunology and hospital epidemiology are particularly subject to this objection. As a result, this book does not fill any of the voids created by standard treatments of a similar nature. Several of the latter will probably be more useful to those who work and live by the jargon and methodology of this side of the Atlantic.

F. ROBERT FEKETY

Surgery of the Aorta and Its Branches. By JAMES D. HARDY. 386 pp., $6.50. J. B. Lippincott Co., Philadelphia, Pa., 1960.

This monograph, originally begun as a series of articles for journal publication, is a readable digest of available information on the growing field of surgery of the aorta and great vessels. It is not a complete treatise on techniques available, although some of the methods used by the author are described in detail. Since this is a particularly productive period in the field of vascular surgery, it is helpful to have such a monograph as a source of reference material. It will be of value to students and surgeons as a survey of this field.

HENRY T. BAHNSON

Cold Injury. Transaction of the Sixth Conference, July 1958. Edited by STEVEN M. HORVATH. 375 pp., $6.50. The Josiah Macy, Jr. Foundation Publications, New York, N. Y.

The last of the multiprofessional conferences on cold injury is reproduced in book form. It provides again a unique and very successful mode of communication between different scientific disciplines.

The participants, all with wide interest and experience in the physiology and effect of cold on lower mammals and man, provide the basis for informal discussions on hibernation, poikilothermia, man in the Antarctic, wind chill, heat production, thermogenesis, metabolic and energy balances, and cold adaptation.

A fundamental insight into a subject which has become very important both from the medical and military points of view is provided. Most discussion is into the physiology of cold and its effect on mammals. Practical effect is also discussed however especially in regard to cold adaptation, wind chill, and survival in a chronically cold environment (Antarctic).

This book with its interesting interchanges between undisputed leaders in the field of cold injury should be read by all who are using hypothermia as a mode of medical treatment, are interested in the physiology of cold, or are interested in the practical effect of cold on man.

JAMES R. JUDE

Gestation. Transactions of the Fifth Conference, March, 1958. Edited by CLAUDE A. VILLEE. 262 pp., $5.75. The Josiah Macy, Jr. Foundation Publications, New York, N. Y.

This important contribution has suffered by the passage of time. In the first place, it appeared late in 1959 or early in 1960. In the second place, procrastination on the part of the reviewer has delayed this evaluation of its contents until three years have elapsed since the conference took place. Much of the information, therefore, has either become well-known (or has been superseded) as the result of subsequent publications by the members of the conference.

The first two presentations represent reports on classical studies of the placenta which are still in progress. As such they represent magnificent reports on knowledge up to 1958; first of the comparative biology of the placentas and placental-like structures of the higher vertebrates and mammals by Amoroso, and second of the circulation in the primate placenta from the brilliant anatomical studies of Elizabeth Ramsey. The physiological studies she and this reviewer have subsequently undertaken have confirmed beyond question the hypotheses presented here.

These classical presentations are followed by a discussion of venous drainage from the uterus by Joseph Bieniarz of Gdansk, Poland. Bieniarz's presentation is largely devoted to the dubious thesis, which he attempts to prove by deductive reasoning, that toxemia of pregnancy is due to high implantation of the placenta. Subsequent events indicate that Bieniarz has yet to provide physiological studies of uterine hemodynamics to support the deductions here reported.

The most fascinating portion of the conference is the report of the University of California veterinarian Louis Holm of the existence of genetically determined prolonged gestation in Holstein-Friesian cattle. In this breed of cattle a dominant-recessive gene pair is responsible for pregnancy being prolonged as much as ninety days past term if the calf is homozygous for the recessive. This state of affairs suggests that Holm's studies in progress may enable us to elucidate the problem of postmaturity in the human which has heretofore been considered as either a myth, or a menace.

The volume concludes with typical Macy Conference discussions of some of the finer points of placental morphology and physiology, the Rh factor, and the effects of stress on the fetal circulation. As is usual with the Macy Conferences such verbatim reports of discussions are distracting to the reader. Students of these subjects will, accordingly, prefer to consult the original papers of the discussants rather than this volume.

GEORGE W. CORNER, JR.

A Bibliography of Internal Medicine. Selected Diseases. By ARTHUR L. BLOOMFIELD. 312 pp., $6.00. The University of Chicago Press, Chicago, Ill., 1960.

This is an extremely interesting, lucid and easily read book. Its small size belies its great importance to the student interested in classic papers about prominent disease entities. Dr. Bloomfield who has written "A Bibliography of Internal Medicine: Communicable Disease" has used the same technique in this present volume. Selecting twenty-one "diseases of old and honorable lineage" he has listed "every reference of fundamental import in the development of each, even to the point of translating French and German classics."

Covered are such syndromes as Stokes-Adams, subacute bacterial endocarditis, pernicious anemia, Hodgkin's disease, Addison's and Graves' disease, to name only a few of the many entities covered.

One of the most important is the section on coronary occlusion and myocardial infarction. Dr. Bloomfield begins with the 1799 Caleb Parry report on "an inquiry into the symptoms and causes of the syncope anginosa" in which the first pathological correlation was made of the role of the coronary arteries in myocardial infarction and the clinical history of angina pectoris.

In a very absorbing series of classic papers, Dr. Bloomfield reviews the tortuous course of further observations and proofs extending from the 1842 John Erichsen explanation of "the influence of the coronary circulation on the action of the heart" and the 1850 discourse of Richard Quain "on the fatty diseases of the heart", to the more modern observations of the 1912 report of James Herrick on "the clinical features of sudden obstruction of the coronary arteries".

Included is the 1918 classic of Fred M. Smith's on "ligation of coronary arteries with electrocardiographic study" and the first published clinical observation in 1920 on "the significance of transient localized pericardial friction in coronary thrombosis", published in the Albany Medical Annals.

In addition papers are reviewed by MacCallum and Taylor, Thomas Lewis, Mallory and White, and Wright's first paper on anticoagulants in coronary heart disease.

Dr. Bloomfield has unearthed other such treasures in the other syndromes mentioned and has put together a compact but extremely fascinating chronological account of classics which make extremely interesting reading for anybody with even a passing interest in these subjects.

PERRY HOOKMAN

Drugs of Choice 1960–1961, 2nd edition. Edited by WALTER MODELL. 958 pp., $13.50. The C. V. Mosby Co., St. Louis, Mo., 1960.

The authors again have come up with a practical guide "to the selection of the best drug for a particular therapeutic problem." In these days of high pressure advertising when each day, the mails are bombarded with a barrage of "medical information" to both patient and physician, a book such as this is sorely needed by the busy practitioner. This is an up-to-date compilation of competent medical opinion of new and old drugs by eminent authorities.

The book is divided into major sections. Each section contains a short discussion of clinical circumstances requiring the use of the drug. There follows a discussion of several drugs and all data as to side effects and other disadvantages. Also important are the many references which form a base for the opinions presented therein.

The present volume, the 1960–61 edition, has been revised from the previous volume 1958–59 after a two year lapse, because as the author explains, "a shorter period would be too brief for a substantial experience with the drugs introduced in the interval". In addition the new volume contains minor but important innovations, one being "the choice of sedatives and tranquilizers", and the other, "the physical and chemical considerations in the choice of drugs". The additions are informative and easily comprehended.

PERRY HOOKMAN

Zabriskie's Obstetrics for Nurses, 10th edition. By ELISE FITZPATRICK and NICHOLSON J. EASTMAN. 571 pp., $6.00. J. B. Lippincott, Co., Philadelphia, Pa.

This volume represents a complete revision of one of the classic textbooks for nurses. Its senior author, Miss Fitzpatrick, now as for several years on the teaching staff of the nursing school at Western Reserve, is well known to several generations of Hopkins Woman's Clinic personnel, where she was once in charge of the Fourth Floor. The reputation of her collaborator requires no comment. These distinguished authors have performed a great service in completely modernizing both the text and the illustrations of this textbook. Two important additions have been made in the chapters on The Mental Hygiene of Pregnancy by Leo Kanner, and on Disaster Insurance for Mothers and Babies by Sara Fetter, John Whitridge, Jr., and Colonel John Welch. The material included here should be read by all those with the slightest interest in maternal and child health.

A reviewer of books, if a specialist in the field covered, can always find a few points to quibble about in the most authoritative of textbooks. Such points always involve either the impossibility of a textbook being absolutely up to date or items of special interest to the reviewer. For example, the thiazide diuretics have multiplied so fast that the information herein is hopelessly out of date. Furthermore, this reviewer feels that the value of progesterone in preventing abortion and premature labor has been virtually ignored. Similarly, the discussion of "Natural Childbirth" does not do proper justice to the Psycho-prophylactic Method which is the modern improvement upon the teachings of Grantly Dick Read.

The criticisms mentioned above, however, do not detract from the value of this text-

book. Fitzpatrick and Eastman have succeeded beyond measure in restoring *Zabriskie's Obstetrics for Nurses* to its rightful place in the forefront of nursing education.

GEORGE W. CORNER, JR.

BOOKS RECEIVED FOR REVIEW

Aids to Physiology, 5th edition, 1961. By E. T. WATERS. 282 pp., $3.00. Bailliers, Tindall and Cox, London. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

The Cardiac Arrhythmias. By BRENDAN PHIBBS. 128 pp., $7.50. The C. V. Mosby Co., St. Louis, Mo., 1961.

Chemicals in Your Food and in Farm Produce: Their Harmful Effects. By FRANKLIN BICKNELL. 192 pp., $2.95. Emerson Books, Inc., New York, N. Y., 1961.

Elementary Human Physiology. By TERENCE A. ROGERS. 417 pp., $6.50. John Wiley & Sons, New York, N. Y.

Essentials of Neurology. By JOHN N. WALTON. 422 pp., $6.75. J. B. Lippincott Co., Philadelphia, Pa., 1961.

Heredity in Ophthalmology. By JULES FRANCOIS. 731 pp., $23.00. The C. V. Mosby Co., St. Louis, Mo., 1961.

Management of Obstetric Difficulties, 6th edition. By J. ROBERT WILLSON. 687 pp., $16.50. The C. V. Mosby Co., St. Louis, Mo., 1961.

The Metabolic Basis of Inherited Disease. Edited by JOHN B. STANBURY, JAMES B. WYNGAARDEN AND DONALD S. FREDERICKSON. 1477 pp., $30.00. McGraw-Hill Book Co., Highstown, N. J., 1960.

Myasthenia Gravis. The Second International Symposium Proceedings. Edited by HENRY R. VIETS. 707 pp., $29.00. Charles C Thomas, Publisher, Springfield, Ill., 1961.

The Practitioner's Handbook. Edited by WILLIAM A. R. THOMSON. 711 pp., $12.50. J. B. Lippincott Co., Philadelphia, Pa., 1960.

CONTROLLED OXYTOCIN INFUSION AS A METHOD OF THERAPEUTIC ABORTION IN EARLY PREGNANCY

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It is generally accepted that abdominal hysterotomy is a safer procedure than a dilatation and curettage if one is to interrupt pregnancy after twelve weeks. The reasons for this can be summarized very succinctly in the words of Eastman (1) who states that "the vaginal operation of therapeutic abortion is fraught with several hazards including danger of perforating the uterus, hemorrhage, retention of placental fragments, and infection. The likelihood of these complications augments sharply after the twelfth week and for this reason the procedure should never be done vaginally when the duration of pregnancy has exceeded that limit. If it has, and if interruption of pregnancy appears urgent, abdominal hysterotomy is the operation of choice." However, even abdominal hysterotomy, though the lesser of two evils, is not the ideal solution and may be associated with an operative and postoperative risk in a patient already in a poor general condition as a result of the disease which necessitated therapeutic abortion. Further, the presence of a scar in the uterus may in future pregnancies render cesarean section the safer way of delivery. It would seem, therefore, that medical induction might be a more desirable procedure. However, in the past, it was thought by many that the uterus during early pregnancy is not sufficiently responsive to oxytocin and thus medical induction might not be possible. Recently we have been using a continuous controlled intravenous infusion of oxytocin, Syntocinon (Sandoz), to interrupt early pregnancies for therapeutic reasons and this report deals with the experiences gained and details of the procedure employed.

Table I gives the summary of four such consecutive cases handled by us. Out of these four cases three were completed spontaneously, while in the fourth (Case 2) the internal os dilated up to one finger, the uterus became firm and smaller, and the abortion could be completed by dilatation and curettage. We consider this a partial success because initially in this case a dilatation and curettage was considered contraindicated due to a soft, boggy uterus, two centimeter long cervix and a closed internal os. In retrospect, on the basis of recent cases, we now feel that this case could also have been completed successfully without a dilatation and curettage had we known at that time that massive doses of Syntocinon (2 to

TABLE I

Summary of Cases

Case No.	Name and History	Initial Cervical Status	Indication	Duration of Pregnancy	Total Induction Time	Total Syntocinon Used	Result and Remarks
1	E.P. JHH #948880 Primigravida	1.5 cm. long, firm. Internal and external os closed	Severe hyperemesis uncontrolled	17–18 weeks	32.50 hours	180 U	Spontaneous expulsion of P.O.C. after artificial rupture of membranes at 3 fingers dilatation of cervix
2	E.G. JHH #801244 Multiparous	Cervix soft, closed, posterior, 2 cm. long	German measles	13 weeks	51.40 hours	3140 U	Internal os dilated to 1 finger and evacuation completed by D & C, although initially D & C was considered contraindicated
3	E.N. JHH #988445 Multiparous	2 cm. long, firm. Internal os closed. External os admitted one finger	Eclampsia superimposed on chronic hypertensive disease	20 weeks	13.02 hours	10 U	Spontaneous expulsion of products of conception
4	J.H. JHH #992388 Multiparous	1.5 cm. long, firm. Internal os closed. External os admitted one finger	German measles	16 weeks	17.50 hours	720 U	Spontaneous expulsion of products of conception after artificial rupture of membranes at 4 fingers dilatation of cervix

4000 mU per min.) can be administered safely with no side effects whatsoever. This statement receives support from the outcome noticed in Case 4 at sixteen weeks of gestation. In this case, the cervix dilated to three fingers from a completely closed status in a matter of six hours only. This could be achieved only with the help of massive Snytocinon infusion in the neighborhood of 1 to 4000 mU per min.

METHOD

The detailed steps of the controlled infusion procedure will be described using Case 1 as an illustration:

E.P. (JHH ※97 88 80), an eighteen year old primigravida, was admitted via the Accident Room with a diagnosis of severe hyperemesis and a weight loss of thirty pounds. Her last menstrual period was September 26, 1960, giving an expected date of confinement of July 3, 1961. After seventeen days of hospital stay she continued to vomit despite the presence of a Levine tube and intravenous fluid therapy. Her general condition started to deteriorate although it did show some improvement initially. Her pulse rate began to show a steady unremitting rise, until by February 3, it was 150 per minute. It now seemed that despite seventeen days of hospitalization with conservative therapy, no headway was being made. The alarming feature was the persistent tachycardia which indicated that interruption of the pregnancy by the least traumatic means would be advisable before the patient's condition deteriorated even further. On the morning of the induction, February 4, the patient was taken to the delivery room. Examination at this time revealed the uterine fundus two fingers below the umbilicus. The cervix was firm, 1.5 cm. long and closed. In order to record the intra-amniotic pressure to control the pitocin infusion, the abdomen was prepared with all sterile precautions, the bladder emptied and a vinyl catheter (0.02″ I.D.) introduced into the amniotic sac through a No. 17 gauge spinal needle. This catheter was then connected to a force and displacement pressure transducer[1] which, in turn, transmitted the pressure variations to a calibrated strain gauge amplifier with direct writing recorder.[2] The whole system of catheter and pressure transducer was filled with normal saline, and with the aid of a three-way stopcock, and a 500 cc. bottle of saline under pressure, the system could be flushed out as and when required. This served to prevent epithelial squames, etc. in the amniotic cavity from blocking the end of the catheter. With this arrangement, the spontaneous activity of the uterus was recorded (Fig. 1).

As will be seen, the tonus is low, 2 to 4 mm. Hg and contraction am-

[1] Pressure transducer Model No. P23DB. Statham Instruments, Inc. of Puerto Rico.
[2] Sanborn Company, Waltham, Mass.

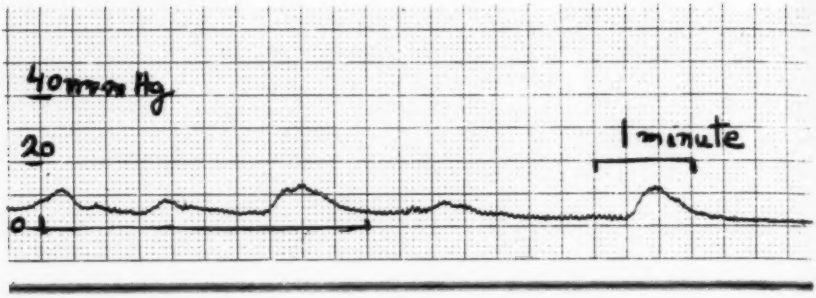

FIG. 1. Spontaneous activity of the uterus. Note minimal tonus, infrequent contractions with low amplitude.

plitude seldom exceeded 10 mm. Hg. The method of administering pitocin is as follows: A vinyl catheter (0.020″ I.D.) was introduced into an antecubital vein for a distance of three to four inches. It was then connected to a 30 cc. syringe placed in the infusion pump.[3] This will deliver a continuous, accurately measured dose of oxytocin and, when necessary, the rate can be varied by adjusting the settings on the pump. In this case, we used Syntocinon (Sandoz) diluted in a 500 cc. bottle of normal saline to which 10 mg. of heparin had been added to prevent thrombosis or clotting in the catheter. It is our practice to start with the lowest dosage, that is, 0.5 milliunits per minute and increase slowly until effective contractions are obtained. Figure 2 shows the contractions obtained four and a half hours after commencement and using a dose of 100 milliunits per minute. Tonus is 4 to 9 mm. Hg and amplitude averages 55 mm. Hg for each contraction with a frequency of six contractions per ten minutes. This gives a value of about 300 Montevideo units.[4] By this time, the cervix was 50 per cent effaced but with no dilatation. The effect of over-dosage can be seen from Figure 3, where 250 milliunits per minute increased the tonus to as high as 25 mm. Hg and contractions were of poor amplitude. When the dose was lowered to 100 milliunits per minute, the tonus fell to normal and contraction amplitude again increased (Fig. 4). Later, the dosage could be reduced gradually to 20 milliunits per minute and still maintained good uterine contractions until the end of the induction. After ten hours, the cervix was fully effaced, but closed. The ensuing twenty-two hours produced slow cervical dilatation until at thirty-two hours and fifty minutes, vaginal

[3] Harvard Apparatus Company, Dover, Mass.

[4] Montevideo unit is defined as the product of frequency per 10 minutes and average amplitude of contraction.

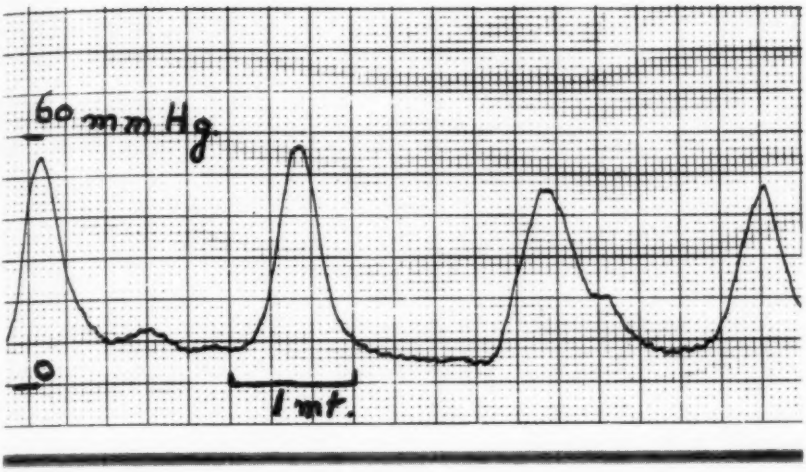

FIG. 2. Uterine contraction after 4½ hours of pitocin infusion. Note good effective uterine contractions.

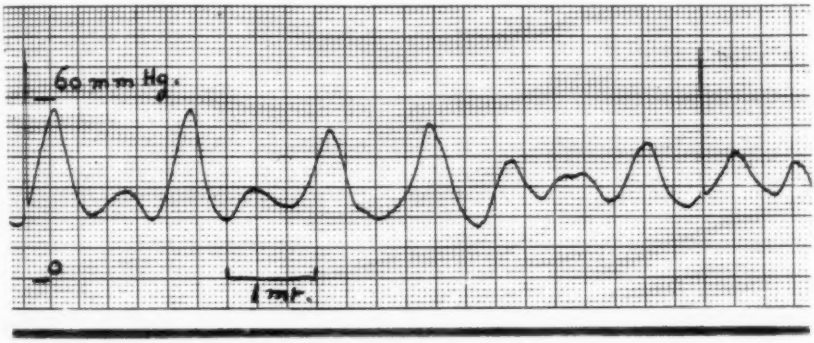

FIG. 3. Note the rise of tonus and somewhat tetanic type of contractions due to over dosage of pitocin infusion.

examination revealed the cervix to be three fingers dilated. The bulging membranes were ruptured and the fetus and placenta were expelled spontaneously in their entirety. The total dose of pitocin given was 180 units Table II shows a summary of progress.

The patient's post-abortum course was uneventful with no signs of sepsis. She partook of a normal diet within three days and was discharged from the hospital nine days after completion of the induction.

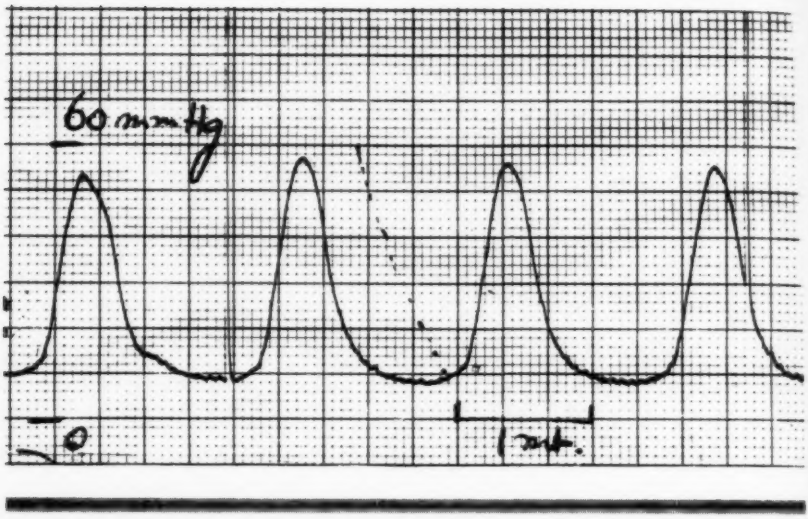

FIG. 4. Note re-establishment of the good effective uterine contractions when dose of pitocin infusion was reduced to optimal levels.

TABLE II

Summary of Progress

Time	Progress
0 hour	Cervix 1.5 cm. long, firm. Os closed, minimal spontaneous uterine activity.
4 hours 30 minutes	Cervix ¾ cm. long, os closed.
10 hours	Cervix fully effaced, os closed.
27 hours	Cervix one finger dilated.
30 hours	Cervix two fingers dilated.
32 hours 50 minutes	Cervix three fingers dilated, artificial rupture of the membranes with complete expulsion of products of conception.

Total Time: 32 hours 50 minutes
Total Syntocinon Used: 180 units

DISCUSSION

The technique is time-consuming and involves the use of expensive equipment. However, it seems justified in these cases as it is devoid of two major hazards encountered in abdominal hysterotomy. In the first place, the immediate operative and postoperative risk is practically nil here, and, secondly, the uterus is left unscarred. We emphasize that the

infusion pump has the advantage over the intravenous drip in that a continuous measured infusion can be administered with much greater accuracy to provide optimum uterine activity. When the occasion necessitates an increase or decrease of dose, as indicated by the recorder, the pump setting can be changed without fear of over or under-shooting. The monitoring of uterine contractions with the pressure transducer and strain gauge amplifier is also essential in that it gives a precise record of what is happening to tonus, contraction amplitude, and frequency. The clinical observer, by palpating the abdomen, can tell frequency, but can only give a vague idea of contraction amplitude and basal tonus in between contractions. Hence, the dosage of pitocin cannot be accurately gauged. This technique obviates these difficulties and the stage is reached when the absolute minimal dose of oxytocin, which gives good uterine contractions effective in dilating the cervix, can be administered. The procedure incapacitates the patient very little. She could move around in bed, sit up and eat meals, if she desired. We have used up to a total of 3140 U of Syntocinon, at a rate at times up to 1 to 4000 mU per minute, with no side effects whatsoever over a period of about fifty hours. We advise not to rupture the membranes artificially as the possibility of retained placenta is greater when it is performed before almost full cervical dilatation, especially at an early stage of pregnancy. It would seem from these cases that controlled oxytocin infusion, monitored by intra-amniotic pressure recording, could be used as the method of choice for emptying the uterus in early pregnancy. At present we accept only one contraindication for using this procedure for terminating an early pregnancy, and that is when pregnancy is complicated with carcinoma of the cervix.

SUMMARY

A preliminary report on controlled oxytocin infusion as a method of choice for therapeutic abortions in early pregnancy has been described. Its advantages over other acceptable procedures, such as abdominal hysterotomy, have been discussed.

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SUBCLINICAL PNEUMOCYSTIS CARINII PNEUMONITIS IN ADULTS

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A subclinical form of pulmonary Pneumocystis carinii infection is described in two adults in whom the presence of the agent in the lung had produced no symptoms. The lesion consisted of a bland, focal interstitial pneumonitis without the typical foamy alveolar exudate and with only small numbers of organisms. The findings were similar to those reported in three infants and in one older child (1) as well as in seven other babies in whom the clinical and morphologic data closely resembled the cases 1, 3 and 4 of the previous study (2).

In its typical form pneumocystis pneumonia consists of pulmonary consolidation with a foamy alveolar exudate containing large numbers of organisms and with a plasma cell and mononuclear cell infiltration of the septa. These changes interfere with gas exchange in the alveoli and, when widespread enough, may cause asphyxiation. Until 1956 it was considered that the disease was virtually limited to early infancy when Hamperl called attention to its occurrence in three adults, raising to six the recorded number of adult cases (3). Since that time fifteen further instances of the infection in adults have been reported (4 to 8) which are tabulated by Rubin and Zak as well as by Hennigar *et al.*, and it seems probable that others exist. The autopsy findings in these patients revealed either a focal or diffuse pneumonic process with foamy alveolar exudate and numerous organisms. Clinically the infection remained unrecognized since in the majority of adults it occurred as a complication of a pre-existing severe disorder.

MATERIAL AND METHODS

The routine hematoxylin-phloxine stained lung sections from recent autopsies of adults dying with either lymphoma or leukemia who had been treated for some time with either adrenal steroids, antimetabolic agents, roentgen radiation and antibiotics or with a combination of these therapeutic regimens, were re-examined for the presence of changes possibly related to the presence of pneumocystis organisms. Among sixty-three autopsies with either leukemia or lymphoma thirty-two cases con-

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formed to the above-mentioned criteria. The lung slides of nineteen autopsies appeared sufficiently suspicious to warrant further study and on the Zenker-fixed lung blocks from these the following stains were done: Giemsa, PAS with digested control, Gomori's methenamine silver-safranine and the MacCallum-Goodpasture modification of Gram's method. Smears of fresh or fresh-frozen lung tissue were not available. The identification of Pneumocystis carinii in section was based on the presence of four, six or eight definite bodies of the proper dimensions and tinctorial characteristics in cysts with the Giemsa stain, a partly Gram-positive reaction in an occasional cyst ("sickle or cup" cyst) (9) and positive reactions with methenamine silver and PAS, the latter also after digestion. In this manner pneumocystis organisms were demonstrated in the lungs of two patients.

CASE 1

A forty-nine year old white male who had previously been in good health, noted in January, 1959 the development of multiple nodules over the posterior aspects of the neck. At another hospital the diagnosis of acute lymphoblastic leukemia had been established on the basis of a lymph node biopsy and of a white blood cell count of 13,000 per cc. mm. with a predominance of lymphobastic cells. Treatment begun on February 1, had consisted of roentgen irradiation to the posterior neck, prednisone, 30 mg. per day, 6-mercaptopurine, 150 mg. per day, and methyltrexate, 5 mg. per day and was continued until August 26 when 6-mercaptopurine was discontinued because of leukopenia and thrombocytopenia while hormone therapy was maintained.

On August 28 the patient was admitted to Emory University Hospital because of fever, malaise and epigastric pain. On physical examination the temperature was 102°F., the pulse rate 130 per minute, the respirations 28 per minute and the blood pressure 110 mm. Hg systolic and 75 mm. diastolic. Several 1 to 2 cm. rubbery posterior cervical, axillary and inguinal lymph nodes were palpable bilaterally. A few white patches consistent with monilia infection were seen in the posterior pharynx. The physical examination was not otherwise remarkable.

On admission the hemoglobin was 9.3 gm. per cent and the white blood cell count 4500 per cc. mm. with 10 blast forms, 9 promyelocytes, 6 band forms, 37 segmented cells, 2 eosinophils, 1 basophile, 33 lymphocytes and 2 monocytes. The urine was acid with a specific gravity of 1.020 and a trace of protein. The sediment showed occasional leukocytes. A routine throat culture yielded only the usual mouth flora. No plasma protein determinations were recorded.

On the third hospital day typical oral moniliasis was said to be present and coarse rhonchi and occasional rales were noted over the chest bilaterally. A chest roentgenogram on admission revealed a diffuse parenchymal infiltrate, moderate bilateral hilar lymph node enlargement and a minimal left pleural effusion and on the fifth day showed extensive fluffy pulmonary densities which were interpreted as edema superimposed upon pneumonitis. At this time treatment with 12 million units of penicillin and 2 gm. of erythromycin per day was begun and 6-mercaptopurine with a dose of 150 mg. per day was reinstituted. The physical and roentgenologic findings continued unchanged. On the seventh day severe pulmonary edema appeared which responded fairly well to treatment. At this time Fungizone clyses (total dose received was 2.5 gm.)

were given since it was thought that the pulmonary lesion might be caused by monilia. On the next day hypotension refractory to treatment terminated in death.

This patient had received in addition to roentgen ray treatment 6360 mg. of prednisone over a period of seven months, 18,050 mg. of 6-mercaptopurine for about three and one-half months and 465 mg. of methyltrexate for about three months.

Autopsy which did not permit examination of the head and neck showed widespread infiltration with lymphoblastic cells involving chiefly the spleen, lymph nodes and bone marrow and to a lesser extent the lungs, gastrointestinal tract, liver, kidneys, and testicles. The combined weight of both adrenals was 16.2 gm. and the organs revealed slight focal nodular hyperplasia and moderate lipid depletion of the cortex. Additional findings consisted of diminished hematopoiesis, pulmonary edema, hydrothorax (100 cc. bilaterally), slight dependent edema and some bronchiectasis in both lower lobes. Bacterial cultures taken from the heart blood and lungs yielded no growth. Sabouraud dextrose agar tubes inoculated with lung tissue were reported as showing "no pathogenic fungi".

The findings of particular interest were confined to the lung. The right lung weighed 1490 gm., the left 1480. The left interlobar fissure was obliterated by dense fibrous adhesions. The pleural surfaces were smooth and glistening and transmitted a red color, particularly in the lower portions. The consistency was rubbery. The cut surfaces were wet and red without areas of consolidation. The terminal portions of the bronchi were somewhat dilated and contained some mucus.

In histologic preparations the alevolar septa throughout showed some proliferation of their lining cells as well as a few large mononuclear cells within their walls (Fig. 1). The alveoli contained slight to moderate amounts of granular eosinophilic material and some strands of fibrin but no foamy or spongy exudate. Moderate numbers of large mononuclear cells and rare binucleated cells together with occasional red blood cells were present in the alveolar lumens, while granulocytes were lacking. Some of the mononuclear cells contained in their cytoplasm traces of brown and black pigment and rarely cellular fragments. Sometimes the alveolar contents were condensed and formed either acellular hyaline membrane-like structures or aggregates composed of mononuclear cells entrapped in an acidophilic matrix. There was no evidence of organization. A few pneumocystis cysts were seen in occasional alveoli. They were present singly or sometimes in small clusters, stained magenta with PAS or black with methenamine silver and occasionally were partly Gram-positive (Fig. 2). Morphologic details of the cysts were best seen in Giemsa stained preparations where sometimes six or eight nuclei of the intracystic organisms could be discerned with the oil immersion lens (Fig. 3). The organisms were present either in the alveolar lumen or in close con-

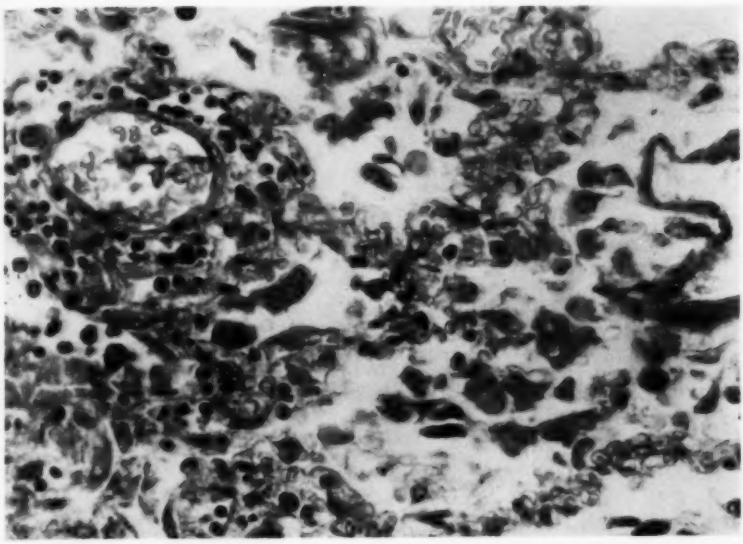

FIG. 1. Pneumonitis with slight mononuclear cell infiltration of septa. The alveoli contain large mononuclear cells and granular material. Giemsa, ×360.

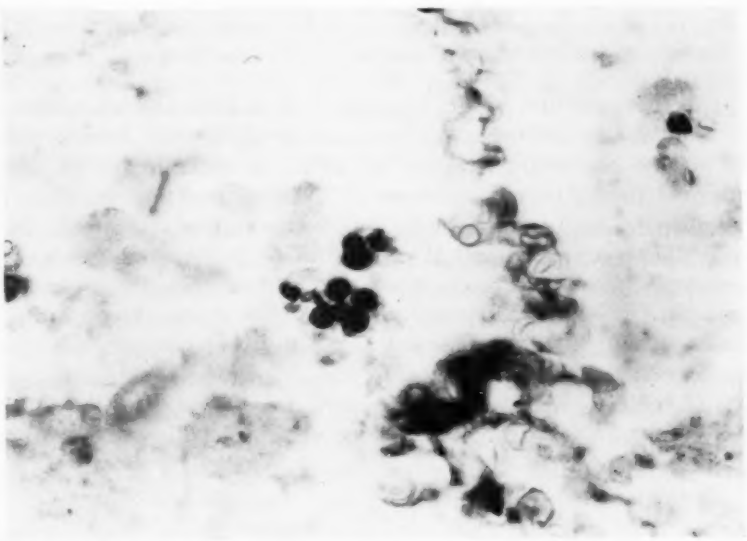

FIG. 2. Small cluster of Pneumocystis carinii cysts (black) in alveolus. Methenamine silver-safranine, ×800.

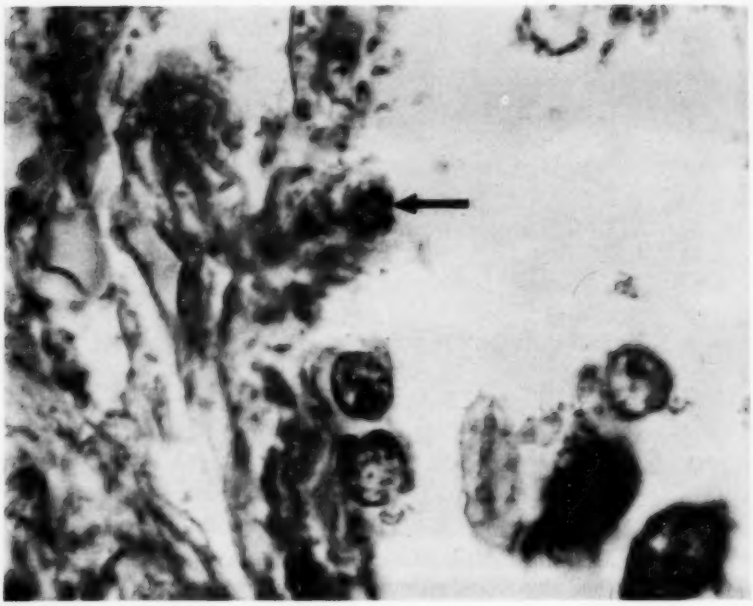

FIG. 3. Pneumocystis carinii cyst (arrow) in close apposition to alveolar septum. Only six of the eight intracystic bodies are visible in this optical plane. For comparison of size note two prominent alveolar lining cells (lower center) and small group of free mononuclear cells in alveolar lumen (right bottom). Giemsa, ×2000.

tact with the septa and commonly were seen in intimate relationship to large mononuclear cells. No free forms of the agent were definitely identified and no convincing evidence of an intracytoplasmic or intraseptal location of the agent was encountered. Neither budding yeast-like nor mycelial structures nor intracellular inclusions such as cytomegalic inclusions or bacteria were found. The appearance of the alveoli containing pneumocystis did not differ appreciably from those without demonstrable organisms. The bronchial epithelium was largely desquamated and some granular acidophilic material as well as mucus were present in the lumen. Some bronchial walls showed fibrosis and slight to moderate amounts of leukemic infiltrate were found in the peribronchial, perivascular, pleural and septal connective tissues. The blood vessels including the septal capillaries were congested.

CASE 2

A fifty-two year old white male enjoyed good health until October, 1957 when recurrent bouts of cramping pain in the lower abdomen appeared. The attacks became more frequent until in January, 1958 an exploratory laparotomy done at another hospital,

revealed a large retroperitoneal mass. A biopsy was taken and the dignosis of undifferentiated lymphoma was made. From then until November the patient was treated at the Winship Clinic of Emory University where he received seven courses of RCo60 teletherapy to various parts of the body either because of bone pain or for treatment of superficial or deep lymph node enlargements. In addition 32 mg. of nitrogen mustard were given when the hemoglobin was 14.4 gm. per cent and the white blood cell count was 4900 per cc. mm. In October treatment with prednisone, 30 mg. per day and with 6-mercaptopurine, 250 mg. per day was begun. The latter drug was reduced to 100 mg. per day after two weeks because of thrombocytopenia and was discontinued in November. At that time four blood transfusions were required to raise the hemoglobin from 7.3 gm. per cent to 10.3 gm. per cent. Except for one normal urine analysis no other laboratory data were recorded for this period.

Because of progressive weakness and persistent anemia the patient was admitted to Emory University Hospital on November 22. The temperature was 103°F., the pulse rate 135 per minute, respirations 22 per minute and blood pressure 122 mm. Hg systolic and 86 mm. diastolic. The positive physical findings consisted of an ill-defined mass in the right upper abdominal quadrant, numerous ecchymoses of the skin and mucous membranes and pallor. The hemoglobin was 7.8 gm. per cent, the white blood cell count 1000 per cc. mm. with 60 per cent segmented neutrophiles, 10 per cent band forms, 10 per cent lymphocytes and 20 per cent monocytes. Platelets were 58,000 per cc. mm. Repeated stool examinations for occult blood were all 4 plus. Urinanalysis showed no abnormal findings. After six transfusions the hemoglobin rose to 10.3 gm. per cent while the white blood cell count ranged between 500 and 1500 per cc. mm. A urine culture taken on December 1 yielded E. coli but multiple blood cultures showed no growth. Total serum bilirubin was 3.9 mg. per cent and the serum alkaline phosphatase was 35.3 Bodansky units. No plasma protein determinations were recorded.

Treatment with prednisone 20 mg. per day was maintained and multiple antibiotics as well as vitamins were also given. The patient left the hospital on December 8 and died suddenly at home two days later.

From October, 1958 until death the patient had received 1640 mg. prednisone. From January, 1958 until death he had been given 12,070 air RCo60 teletherapy over various sites.

Autopsy, which did not include permission for examination of the head and neck, revealed a stem cell lymphoma with widespread involvement of lymph nodes, spleen, bone and bone marrow, liver, pancreas, gastrointestinal tract, kidneys, pleurae and leptomeninges of the lumbar spinal cord. The stomach contained 300 cc. of black, guaiac positive material and showed in the midportion of the greater curvature a 2 cm. active, chronic peptic ulcer which had perforated the gastric wall but was sealed by adherent omentum. This together with the enlarged lymph nodes accounted for the mass noted on physical examination. There was no peritonitis. Additional findings consisted of bronchopneumonia (hemolytic Staphylococcus aureus, coagulase positive and enterococcus), an enlarged liver (1816 gm.) with canalicular cholestasis, icterus, slight bronchiectases, some pulmonary atelectasis, hydrothroax (200 cc. bilateral), diverticula of

the colon and a scarred, obliterated appendix with fibrous peritoneal adhesions. The combined weight of the adrenals was 22.5 gm. and the organs showed marked lipid depletion and slight nodular hyperplasia of the cortex. The blood culture yielded enterococcus. Sabouraud dextrose agar tubes implanted with lung tissue were reported as showing "no pathogenic fungi".

The right lung weighed 475 gm., the left 390 gm. The pleural surfaces were smooth and glistening, and over the left lower and right middle and lower lobes transmitted a dark red color. The consistency of these lobes was rubbery with small, ill-defined areas of firmer consistency on the right. The cut surfaces exuded some foamy fluid and were red with somewhat raised, granular, grey areas 3 to 5 mm. in diameter in the right middle and lower lobes. The bronchi were slightly dilated in their terminal portions and contained much thick, brown-tinged mucus. The bronchial mucosa throughout was thickened and red. The vessels were congested.

Histologic study revealed focal areas where the bronchi and adjoining alveoli contained masses of neutrophilic granulocytes, some large mononuclear cells and small amounts of granular acidophilic material. There were many Gram-positive cocci in clusters and short chains. Many organisms were present intracellularly. Yeast forms, mycelia or intracellular inclusion bodies were not encountered. In other areas the alveolar septa showed some proliferation of their lining cells and minimal infiltration by mononuclear cells including a few cells consistent with young plasma cells. The alveoli were partly collapsed and in general contained moderate numbers of large mononuclear cells. These were rarely binucleated, often contained traces of brown and black pigment or cellular debris and occasionally displayed a somewhat vacuolated cytoplasm. Small amounts of granular acidophilic precipitate were also present. In some of these alveoli and in a few of those filled with granulocytic exudate prolonged search revealed rare pneumocystis cysts either singly or in small aggregates. The cysts were more easily seen in preparations stained with either PAS or methenamine silver and were rarely in part Gram-positive. In Giemsa stained preparations careful examination with the oil immersion lens disclosed a few cysts in which four, six or eight intracystic organisms could be seen. No free forms of the agent were definitely recognized. The cysts were frequently in close relationship to large mononuclear cells and were located either in the alevolar lumen or against the septa without convincing evidence of either intracytoplasmic or intraseptal location. The bronchial epithelium and walls in the areas of bronchopneumonia were involved in the acute inflammatory process. Elsewhere the bronchial walls showed some fibrosis. There was no appreciable lymphomatous infiltration. The vessels including the capillaries were congested.

COMMENT

These findings indicate that the subclinical form of pulmonary pneumocystis infection occurs in adults where its morphology is similar to the subclinical form of infants. The lesions were apparent only on histologic examination and consisted of small foci where the alveoli were partly collapsed, showed slight mononuclear and plasma cell infiltration of the septa and some proliferation of their lining cells and contained small to moderate numbers of mononuclear or rarely binucleated phagocytic cells, some granular acidophilic precipitate but no foamy exudate and rare pneumocystis organisms. The cyst forms only were definitely identified. They were present either singly or in small aggregates and were located either in the alveolar lumen or in close apposition to the septa. There was no convincing evidence of either intracytoplasmic or intraseptal location but a close relationship of the organisms to either the proliferating alveolar lining cells or to the mononuclear cells free in the alveolar lumen were frequently noted. Neutrophilic granulocytes which were present in the foci of acute bacterial bronchopneumonia in the second patient showed no affinity for the cysts in those areas where the two processes overlapped. In the first patient in whom at autopsy no bacteria were demonstrated in the lung by cultures or histologically, pneumocystis organisms were present in only a few areas while the alveoli throughout contained granular or sometimes condensed acidophilic material and mononuclear cells. Therefore this tissue response in the lung while suggestive was not indicative of the presence of a few pneumocystis organisms.

In contrast to massive pneumocystis pneumonia where the alveoli contain a foamy exudate teeming with the free and cystic forms of the agent, the histologic identification of the organism in tissue sections is difficult in the subclinical form where only a few cysts can be seen in occasional and scattered alveoli. It is probable that rare free forms of the parasite are present in these areas but their identification in tissue sections is too uncertain for diagnosis. All forms of the agent are best seen in smears from fresh or fresh frozen tissue. When smears are not available the PAS and methenamine silver stains are useful in locating the organism. These methods stain the envelope of the cyst but do not afford the visualization of its internal structure. For this purpose the Giemsa stain is valuable since it reveals such morphologic details as the tinctorial characteristics, arrangement and number of the intracystic bodies, but it must be realized that the appearance of the organisms in fixed tissue sections differs somewhat from that seen in smears.

This study does not clarify the nature of the agent but indicates again

that latent pulmonary pneumocystis infection exists in adults as well as in babies and older children (1). The observations of Weisse and Wedler suggested that latent infection of man is not uncommon (10). These authors studied with histologic preparation and imprint smears the lungs of 152 unselected autopsies of which 125 were adults between the ages of twenty-one and seventy-nine years while the remaining twenty-seven cases represented the ages from five days to twenty years. Rare pneumocystic organisms, not associated with pneumocystis pneumonia were found in twenty-three cases of which ten were encountered among the 125 adults while of the remaining thirteen cases eleven occurred in infants between the ages of five days and seven months and one each at four and five years respectively. These patients had died with a variety of diseases which did not include a single instance of "interstitial plasma cell pneumonia". Robinson found only two instances of the infection in 203 autopsies performed on males between the ages of twenty-four and eighty-five years (7). In both cases pneumocystis pneumonia with foamy intra-alveolar exudate appeared to be present and the process resembled the findings in other adults with this infection rather than those encountered in the present material or as previously reported in infancy and childhood.

In the majority of adults including the present patients, pneumocystis infection occurred as a complication of a protracted illness often treated for long periods of time with steroids, antibiotics, antimetabolic agents or radiation (4). The only apparent exceptions were two patients, one studied by Anderson and Barrie (5) whose liver revealed a "mild cirrhosis of uncertain origin" without clinical or laboratory evidence of hepatic injury and another reported by Hennigar *et al.* (8) in whom no pre-existing disease was found. In the majority of infants massive pneumocystis pneumonia presented itself as a primary disease although in some instances the infection appeared to be secondary to hypo- or agammaglobulinemia or steroid therapy (11, 12, 13, 14). The subclinical form of the infection in infancy which in its morphologic appearance resembled the present findings, was encountered in apparently normal, healthy babies who had died suddenly without previous illness (1).

It appears that the agent Pneumocystis carinii is of low virulence and while able to infect man, does not find an environment suitable for massive proliferation in the normal human host which at the same time often cannot completely suppress the organism. A similar situation probably exists in other hosts as shown by the experimental activation of a naturally occurring, latent infection in the rat and rabbit (15, 16) and by the finding of occasional organisms in the lungs of many other species in the absence of significant lesions (17). The slightly greater frequency with

which Weisse and Wedler (10) found rare pneumocystis organism in infants and children as compared to adults might indicate that the agent persists more readily in the lung of infants than of adults.

The factors which transform inactive and latent pneumocystis infection into an active and spreading disease are not understood and their mechanisms of action are virtually unknown. The occasional association of gamma-globulin deficiency with pneumocystis infection suggests that an interference with antibody formation or function can create conditions suitable for the unchecked proliferation of the agent. It is unlikely that impairment of antibody is the principal predisposing factor since in the majority of cases encountered in infants and in some adults plasma protein determinations showed no changes. Furthermore the pulmonary lesions in most patients, particularly in infants, showed a plasma-cell infiltration, a finding which has given the disease its original designation, namely interstitial plasma-cell pneumonia, and which is incompatible with hypo- or agammaglobulinemia where these cells are appreciably decreased in number or absent (18). The occurrence of pneumocystis infection in patients with protracted illnesses many of which had received forms of therapy known to interfere with the inflammatory response suggests that an impairment of cellular defenses may also predispose to the acquisition of the disease or to the activation of a latent infection. Since at this time the nature of the agent, its constituents and growth requirements are unknown one can only form tentative ideas as to the type of cell which in the normal host might be able to keep the agent in check. In histologic preparations the granulocytes have never been noted to show an affinity for the organism. Although pneumocystis does not appear to be an intracellular parasite, its frequent and close relationship to large mononuclear cells as seen in both smears and tissue sections (16) might indicate that it is the impaired function of the mononuclear phase of the body defenses which renders the host susceptible to this infection.

It is concluded that subclinical pulmonary pneumocystis infection occurs in adults and may not be infrequent in patients in whom protracted illness and therapeutic measures have impaired antibody and cellular body defenses. The infection may present itself as a widespread pneumonia with manifestations which dominate the clinical picture or may be of minimal extent without noticeably complicating or altering the preexisting disorder. The proliferation of the agent in tissues appears to reflect a derangement of host resistance rather than the virulence of the parasite.

SUMMARY

A subclinical form of pulmonary Pneumocystis carinii infection is described in two adults one of whom died with lymphoblastic leukemia,

the other with stem cell lymphoma. Both patients had received prolonged treatment with large amounts of adrenal steroids as well as with anti-cancer drugs and radiation. The lesion consisted of a slight focal interstitial pneumonitis with some infiltration of the septa by mononuclear and plasma cells and with some large mononuclear cells and small numbers of pneumocystis organisms in the alveoli. There were no foamy alveolar contents. These changes neither produced noticeable clinical manifestations nor did they alter appreciably the pre-existing, long standing disease which seemed to represent an essential predisposing factor in the development of the infection. The findings suggest that following prolonged and severe impairment of host resistance adults may develop pulmonary lesions associated with Pneumocystis carinii either through the activation of a latent infection or by the acquisition of the agent from the environment. Available evidence indicates that pneumocystis is not a pathogenic agent in normal man and therefore, the demonstrable proliferation of the organism in tissues is significant primarly as a reflection of a major derangement of host resistance.

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CHANGES IN THE FINE STRUCTURE OF HeLa CELLS IN RELATION TO GROWTH[1]

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HeLa cells were obtained from a human epidermoid carcinoma of the cervix uteri and established as a tissue culture strain by Gey in 1951 (1). Many reports are available on the biochemical, metabolic and enzymatic properties of HeLa cells, but little is known of their morphology, especially at the electron microscope level. The electron microscopic observations on HeLa cells thus far published are concerned in fact with pinocytosis (2), globoid structures (3), ingestion of colloidal gold (4) and with the effect of cooling (5). In view of the limited fields covered by these investigations, it was thought that a more extensive electron microscope study of HeLa cells might be of interest. We decided to study the fine structure of HeLa cells at different intervals (24, 48, 72 hours) after medium renewal with the purpose of finding whether any of the cell components shows a definite series of changes with passage of time.

MATERIALS AND METHODS

HeLa cells, kept in stock in this laboratory since primary explantation on February 8, 1951, were grown on collagen, on glass, or in clot, with a fluid medium composed of 50 per cent human cord serum and 50 per cent balanced salt solution (Gey). Cultures were grown in roller tubes at 37°C. in equilibrium with air.

Cultures were fixed in the tube with 1 per cent OsO_4 in Acetate-Veronal (pH 7.5) at 4°C. for 3 to 5 minutes after discarding the medium and rapidly rinsing with balanced salt solution at 37°C. Cultures were dehydrated rapidly in a graded series of alcohols and embedded in a mixture (1:9) of methyl-n-butyl methacrylate, containing 2 per cent Lucidol. Polymerization of methacrylate was obtained in the oven at 47°C. Sections were cut with a glass knife in a Porter-Blum (Servall) microtome and examined in a Siemens Elmiskop I at initial magnifications of 1600× to 18000×.

Glycogen in thin sections for electron microscopy was stained with lead hydroxide as used by other investigators (6, 7, 8, 9) in electron microscope studies of glycogen in a number of normal cell types. Saliva digestion (2 hours at 37°C.) was performed with sections of glycogen-containing cells prior to treatment with lead hydroxide in order to check the specificity of the staining.

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A number of sections were stained with $KMnO_4$, following the technique proposed by Drochmans (10) for the demonstration of liver glycogen in electron microscopy. The results with $KMnO_4$ were superimposable to those with lead hydroxide. Saliva digestion abolished the staining of glycogen by both lead hydroxide and $KMnO_4$.

In order to identify more precisely glycogen in the electron microscope image, thick (2μ) sections immediately adjacent to thin sections examined in the electron microscope were stained with the PAS method. The comparative study of thin and thick sections thus prepared showed that the areas rich in glycogen particles stained by lead hydroxide in the electron microscope coincided with the PAS-positive areas in the light microscope. Saliva treatment abolished the staining of the glycogen deposits by the PAS method in thick sections.

The "amount" of glycogen was estimated by evaluating the number of the glycogen-containing cells and the size of the glycogen deposits in thick sections stained with the PAS method. Two cultures for each time interval were used for this "quantitative" approach and in sections from each 100 cells were counted. These were divided arbitrarily into four classes with respect to glycogen content, *i.e.* no glycogen, low, medium, and high glycogen content. The cells with high glycogen content were predominant and the cells with no glycogen were the least numerous in twenty-four hours old cultures. In contrast, the cells without glycogen and with a low amount of glycogen were the most numerous in forty-eight and seventy-two hours old cultures. It should be added, in this connection, that a clear-cut decrease in glycogen appeared to occur in the interval between twenty-four and forty-eight hours after the medium renewal, whereas no clear-cut difference was apparent in the interval between forty-eight and seventy-two hours. Though the method for the estimate of the "amount" of glycogen is only approximate, it seems sufficiently indicative for the purpose of the present investigation.

The material on which this presentation is based includes 4 cultures fixed at twenty-four hours, four cultures fixed at forty-eight hours and seven cultures fixed at seventy-two hours after medium renewal.

OBSERVATIONS

A. Form and Organization

HeLa cells may be spindle-shaped, oval, or round. Microvilli represent a remarkable feature of their surface and appear more prominent in solitary cells, *i.e.* in cells which are not in contact with other cells. Solitary cells are remarkable not only for the high number of microvilli, but also for finger-like, large, protoplasmic projections, whereby the cell may exhibit a stellate form. Usually HeLa cells do not show a definite tendency to form a properly organized tissue, but are rather arranged in a disorganized pattern, which is evident even when cells are in contact with each other. The rarity of desmosomes (see below) may also be taken as a clear indication of the lack of organization in HeLa cells.

B. Nucleus

Nuclei vary in morphological details and in size from cell to cell. The nucleoplasm may consist of an apparent finely fibrous or granular ma-

terial either loosely or tightly arranged, with areas of different electron density even in the same cell. The chromatin appears either concentrated in a single area, close to the nuclear envelope, as well as in the center of the nucleus, or divided into multiple small sized aggregates.

Nucleoli also display variations from cell to cell in the localization and in the organization of the granular component. The latter may be loosely arranged and oriented into a coiled thread-like structure, which obviously corresponds to the nucleolonema (11, 12). The filaments of the nucleolonema are separated from one another by clear, empty spaces. In other cells no particular orientation of the granules can be recognized and thus the whole nucleolus appears as a very dense body, most frequently round in form. From one area at the periphery of the nucleolus a string of granules extends occasionally toward, and makes contact with, the nuclear envelope.

The nuclear envelope does not depart in structure from that described in the normal cell types. Pores in the nuclear envelope are evident in HeLa cells. It is also evident that the nuclear envelope is continuous in some instances with the cisternae of the rough endoplasmic reticulum (ER) in the cytoplasm. A frequent finding in HeLa cells is the presence of numerous deep invaginations of the nuclear envelope, which may appear as "inclusions". Similar observations have been made in normal and in other tumor cell types (13). Since the cytoplasm included in these invaginations may contain different cell components, the nuclear "inclusions", which are actually segregated from the nucleoplasm by the nuclear envelope, may show variable morphology. We have seen also a glycogen containing "inclusion" in the nucleus.

C. Cytoplasm

The amount of glycogen in the cytoplasm decreases with time. This is shown in Figures 1 and 2, taken from twenty-four hours and forty-eight hours old cultures.

The glycogen deposits in cells from twenty-four hours old cultures are so large in size that glycogen may be reasonably regarded as a major component. Cells may in fact contain two or three large deposits of glycogen which extend continuously from the nuclear envelope to the plasma membrane. The areas occupied by the other cell organelles (mitochondria, Golgi, ER, etc.) are by far less extensive than the glycogen areas. The decrease in glycogen content appears to be associated with a difference in the distribution of the cytoplasmic organelles (e.g. mitochondria). In glycogen rich cells the organelles are in fact mostly concentrated in a few areas, either in the vicinity of the nuclear envelope or of the plasma mem-

brane, whereas in non-glycogen containing cells the organelles are more or less uniformly distributed.

In unstained sections the glycogen deposits are characterized by low density and by a diffuse mottled appearance which have been found in glycogen deposits in other cell types, both normal (8, 11, 15) and neoplastic (16). In sections stained with lead hydroxide glycogen appears in the form of particles (Fig. 3) averaging 230 A. in size.

Glycogen does not show any consistent association with the known cytoplasmic systems, regardless of whether it is present in large or in small amounts. Our previous statement (17) that glycogen is associated with the smooth form of the ER, would appear, therefore, to be incorrect. This resulted from observations on some inadequately fixed preparations of twenty-four hours old cultures. It is known that poorly fixed preparations of glycogen-containing tissues give the impression of a finely divided membranous system between the glycogen particles.

The smooth ER is evident in HeLa cells in the non-glycogen containing areas. It does not appear to be prominently developed and it consists, as in other cell types, of tubules and vesicles, continuous with each other and with a branching character.

The rough ER changes from cell to cell especially in regard to the morphology, the extensiveness and the organization of its vesicular and tubular components. Generally the ER appears to consist of round or oval vesicular elements which are scattered in every part of the cytoplasm without any sign of regular organization. Less frequently the ER is represented by cisternae, which may be either single or multiple. When multiple, the cisternae may show a tendency to be oriented into parallel rows (Fig. 4), the whole organization being reminiscent of the organization of the ER in highly specialized cell types.

The ribonucleoprotein particles free in the matrix, *i.e.* unattached to the membranes of the ER, are usually present in high number in HeLa cells and may be organized into rosettes (Fig. 5), as observed by others in different neoplastic cells (18).

Mitochondria are fairly numerous in HeLa cells. In cells without degeneration generally the mitochondria exhibit a relatively small number of cristae and a matrix of low density. Other notable features of the mitochondria are the variations in form and in size from cell to cell. The longest cylindrical mitochondria measure 2.25 μ in length and 0.23 μ in diameter, whereas the largest roughly spherical mitochondria measure 1.00 μ in both dimensions. In the latter mitochondria the cristae are irregularly oriented and the internal chamber is divided, therefore, into remarkably irregular compartments. In cells with clear signs of degenera-

tion the mitochondria show a discontinuous limiting membrane, a highly dense matrix and only remnants of cristae.

The Golgi complex (Fig. 6) in HeLa cells is usually well developed. It consists of horseshoe-shaped arrays of flattened sacs and of round, small vesicles. Swarms of vesicles from the Golgi area tend usually to extend for some length into the surrounding cytoplasm. The juxtanuclear region is the usual localization of the Golgi complex, but representative elements of this system can be found also in cell areas other than the juxtanuclear region. In these circumstances it is common that the differently localized parts of the Golgi system do not appear as separate entities, but rather as continuous with each other, the continuity being established by swarms of small vesicles.

Multivesicular bodies are common components of HeLa cells. Multivesicular bodies (Figs. 3 and 5) consist of an agglomerate of vesicles enclosed by a membrane and are similar to the multivesicular bodies found in other cell types (19, 20). Their diameter in HeLa cells varies from 400 mμ to 700 mμ. The membrane limiting the body is at times discontinuous. In these instances the vesicles inside the body are low in number and are less tightly packed; vesicles with identical characteristics become, however, more numerous on the external surface of the limiting membrane and in its immediate vicinity.

Tonofibrils are uncommon components of the HeLa cells. More frequently the tonofibrils consist of few, short tonofilaments. In a few instances, such as in Figure 7, however, the tonofibrils become more prominent and represent a major feature of the cytoplasm.

Desmosomes are also found in rare instances in HeLa cells. They are very simple in structure. They appear to consist of a simple thickening of the cytoplasmic side of the plasma membrane in the area of contact between adjacent cells (Fig. 8). Instances have been found where the desmosomes appear associated with tufts of filaments which run for a short distance into the cytoplasm, whereas in other instances an association between filaments and desmosomes was lacking.

DISCUSSION

The observations made in this study indicate that glycogen is one of the major components of the HeLa cells in twenty-four hours old cultures and that the decrease in glycogen is the most definite change which occurs with an increase in passage of time.

The overall picture of the glycogen areas in HeLa cells, as seen in low power electron micrographs of unstained sections, is similar to the glycogen areas in normal cell types (8), in the normal (14, 15) and in the neo-

plastic (16) epithelium of the cervix. In these cells, however, the glycogen deposits are less extensive than in HeLa cells in twenty-four hours old cultures.

The size (230 A.) of the glycogen particles, as measured in lead hydroxide stained sections, is within the range found by Revell *et al.* (8) in normal cell types.

Since glycogen is a regular component of the normal cervical epithelium, it is obvious that HeLa cells preserve the glycogen storing ability of the cells from which they are derived. That this ability is stimulated by the quality of the medium is suggested by the unusually large size of the glycogen deposits in HeLa cells in twenty-four hours old cultures. Evidence that the quality of the medium is a factor in the amount of glycogen stored by HeLa cells has been provided by Wu (21). Wu also demonstrated that glycogen is the main source of endogeneous glycolysis in HeLa cells, in which Racker, Wu and Alpers (22) have found intense activity of glycolytic enzymes. In view of these results, the decrease in glycogen content with time in the present investigation may reflect the high glycolytic properties of HeLa cells. The decrease in glycogen content suggests also that glycogen is utilized as a source of energy for cell growth, which is known to occur in the present experimental conditions.

The main characteristics of the ER in HeLa cells are the remarkable variations in morphology and extensiveness of its vesicular components and the lack of a regularly oriented architectural pattern. These characteristics have been found in other neoplastic cells (18) and are in contrast with the characteristics of the ER in normal cell types. It is known, in fact, that the ER under normal conditions in a given cell type shows a characteristic pattern of morphology, extensiveness and organization. However, the ER is present in a very simple form in embryonic cells and increases in prominence and in complexity along with differentiation (23). The variations in the ER from cell to cell in HeLa may, therefore, reflect variations in the degree of differentiation of this system and these variations are known to occur in neoplastic cells.

The mitochondria of HeLa cells are noticeable for variations in form, size and internal structure (*e.g.* length, orientation and number of cristae, density of matrix). Some of these changes may be regarded as a result of degeneration, for they are associated with manifest degenerative changes in other cytoplasmic organelles and in the nucleus. However, motion pictures of cells *in vitro* (24, 25) show remarkable shifts in position of mitochondria accompanied by changes in shape and in size, and these may account for some of the changes in the mitochondria of HeLa cells.

The Golgi complex is remarkable in HeLa cells for being well de-

veloped and for the high number of small vesicles it contains. The Golgi complex is regarded as playing an important role in secretion (26), and in the circulation of intravesicular material, which is conspicuous in cells in active pinocytosis. We consider that the large volume of the Golgi complex and the high number of microvesicles in HeLa are related to pinocytosis; this will be presented in more details in another note (27).

The relatively uncommon presence of tonofibrils in HeLa cells is an indication that they retain to some extent the tonofibrils synthesizing activity of the normal cells from which they originated. The fine structure, as seen with the electron microscope, of the tonofibrils in the normal cervical epithelium and in carcinomas of the cervix *in vivo* has been reported by Hinglais-Guillaud (15) and, respectively, by Luibel *et al.* (16). Brody (28, 29, 30) has recently emphasized that the tonofibrils play a part in the formation of keratin in skin epithelium. The tonofibrils in normal squamous epithelia terminate in desmosomes (31, 32, 33). Association between tonofibrils and desmosomes is not a constant feature of HeLa cells. In HeLa cells, moreover, desmosomes have been found only in rare instances. Desmosomes are usually regarded not only as an anchor point for tonofibrils, but also as specialized areas for the maintenance of cell contact. The rarity of desmosomes as well as the loss of their association with tonofibrils have also been reported in new growths from squamous epithelia, such as skin (34) and cervix uteri *in vivo* (35). The low number of desmosomes appears as an obvious morphological expression of the reduced adhesiveness in cancer cells (36). It is of interest, in this connection, that the fine structures of the desmosomes in the normal cervical epithelium *in vivo*, as studied by Karrer (37), is more complex than the structure of desmosomes in HeLa.

SUMMARY

1. HeLa cells fixed twenty-four, forty-eight, seventy-two hours after medium renewal have been studied with the electron microscope.

2. HeLa cells in twenty-four hours old cultures are characterized by large glycogen deposits which decrease in cells of older cultures. The decrease in glycogen with time suggests that glycogen is utilized as a source of energy for cell growth.

3. The vesicular elements of the endoplasmic reticulum in HeLa cells lack a regular pattern of organization and vary in form and in extensiveness from cell to cell. These variations are regarded as representative of varying stages of differentiation of the endoplasmic reticulum.

4. The Golgi complex is highly developed in HeLa cells. Swarms of small vesicles which extend into the adjacent cytoplasm are conspicuous

in the Golgi complex suggesting that it is involved in the circulation of material engulfed by pinocytosis.

5. Multivesicular bodies are a common component of HeLa cells. The significance of these bodies is obscure at the present time.

6. Tonofibrils and desmosomes are the most uncommon components of HeLa cells. Desmosomes are less complex in structure than desmosomes in normal squamous epithelia *in vivo*.

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PLATE I

FIG. 1. Low power electron micrograph of a HeLa cell from a 24 hours old culture. Glycogen (Gl), which appears as a material of low density, occupies the largest part of the cell body. The nucleus is not included in the plane of section. The central portion of the cell is occupied by a medium dense matrix in which are embedded mitochondria (M). The paucity of organelles in the glycogen areas should be noted. ×4900.

FIG. 2. Low power electron micrograph of a HeLa cell from a 48 hours old culture. The striking difference between this cell and the cell in Fig. 1 is obviously the absence of large glycogen deposits. Mitochondria (M) are more uniformly distributed. Nucleus and nucleolus are indicated by N and, respectively, by n. ×4900.

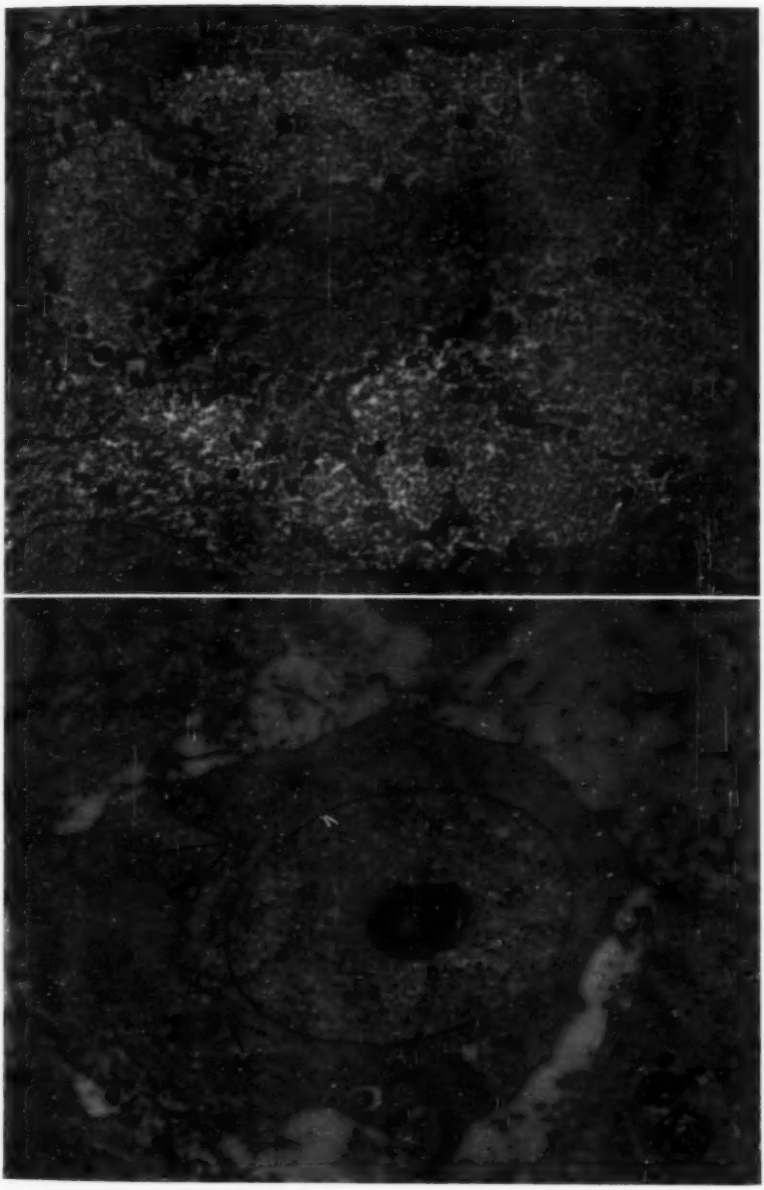


PLATE II

FIG. 3. This micrograph, taken from a lead hydroxide stained section, shows two aggregates of densely stained glycogen particles in a cytoplasmic area in proximity to the plasma membrane (PM). A multivesicular body is indicated by MVB. Besides mitochondria vesicles of the endoplasmic reticulum are present, both round and elongated, in some cases associated with ribonucleoprotein particles (ER). ×25000.

FIG. 4. This micrograph includes the boundaries of two cells, whose plasma membranes (PM) show an extensive area of contact. Microvilli (Mv) are seen in the area where the cells are pulled apart. The cell at the left contains an array of 5 profiles of cisternae (Ci) of the endoplasmic reticulum with attached ribonucleoprotein particles. This represents the highest degree of differentiation found in the endoplasmic reticulum of HeLa cells. Ribonucleoprotein particles free in the matrix are evident in both cells. ×45000.

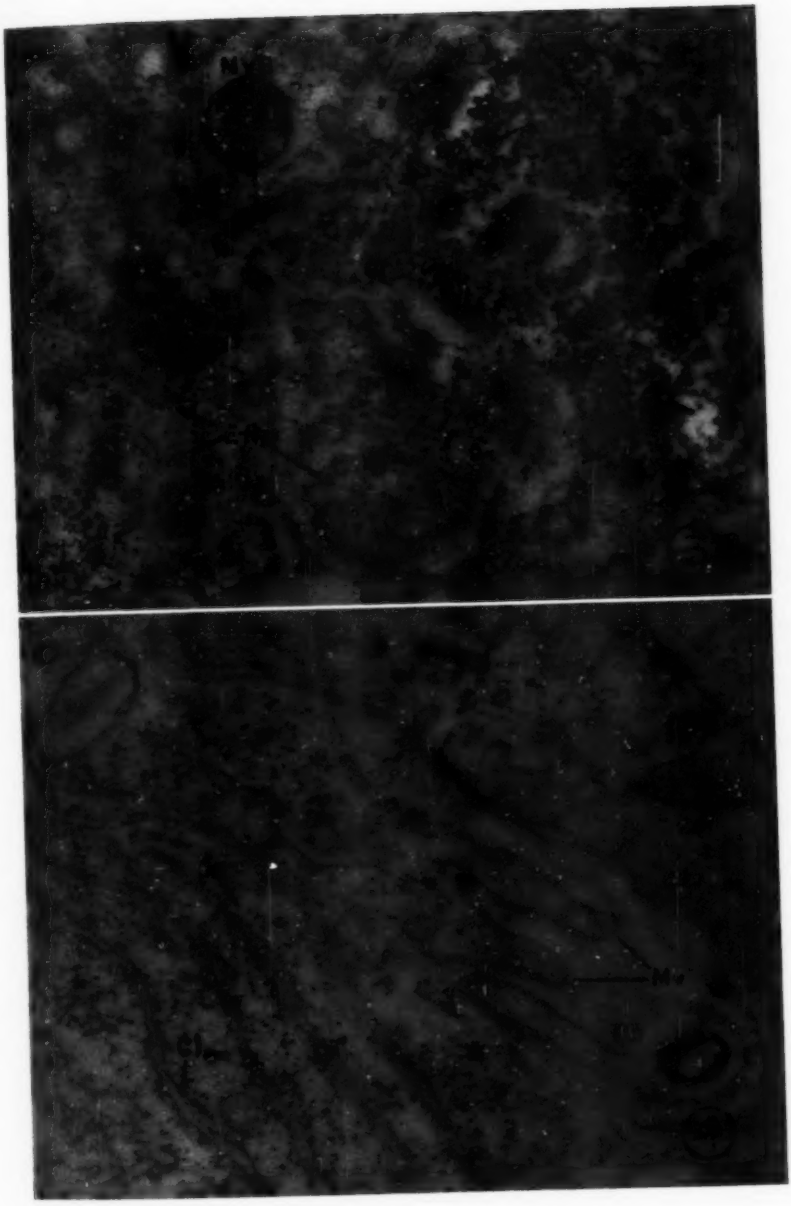

N
M

PLATE III

FIG. 5. Portion of the cytoplasm near the nuclear envelope (right upper corner). Profiles of mitochondria (M) differ from one another in form and size. The number of mitochondrial cristae also varies. There are ribonucleoprotein particles conspicuous in the matrix, at one site aggregated into a rosette (arrow). ER indicates vesicular elements of the endoplasmic reticulum associated with ribonucleoprotein particles; MVB indicates a multivesicular body. ×32000.

FIG. 6. Golgi complex. Here it consists of flattened sacs and of small vesicles, which are also found at some distance from the sacs. ×46000.

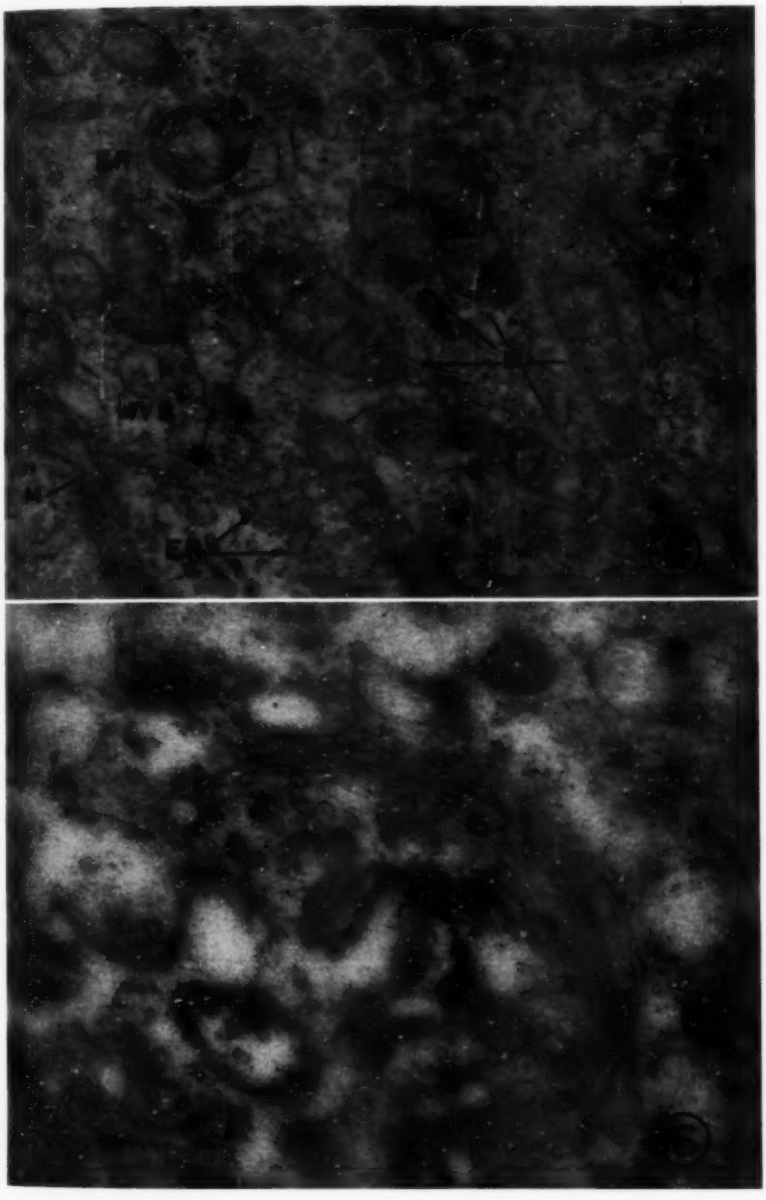
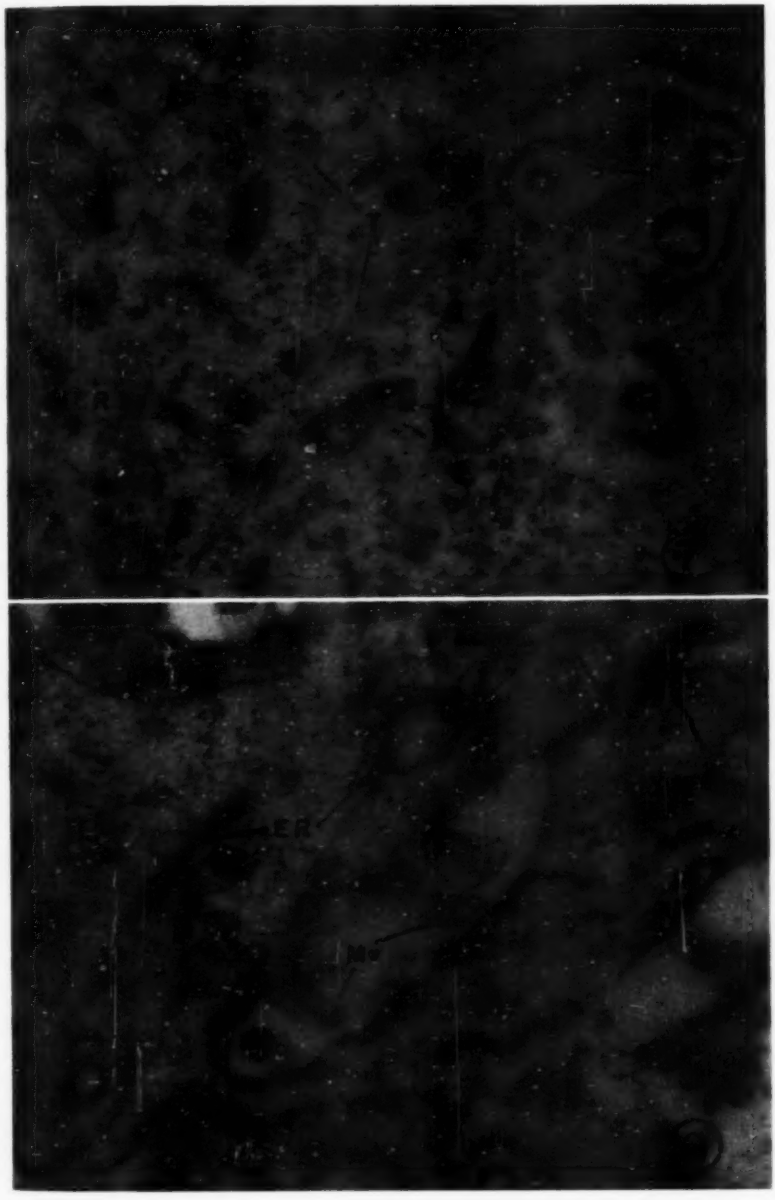
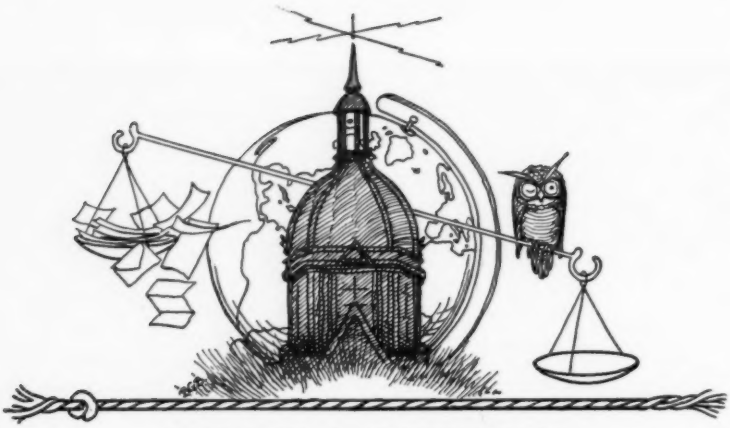


PLATE IV

FIG. 7. Part of a cell near the nucleus (N). Tonofibrils (T) from a cytoplasmic region near the right upper corner run in opposite directions. Mitochondria (M) and ribonucleoprotein particles, either free or attached to vesicles of the endoplasmic reticulum (ER), are readily identified. ×30000.

FIG. 8. Peripheral portions of two adjacent cells. Three desmosomes (arrows) are present in the areas of contact. Microvilli (Mv) appear in the intercellular space. Both cells contain vesicles of the endoplasmic reticulum (ER) and a high number of ribonucleoprotein particles. A mitochondrion is indicated by M. ×46000.





BOOK REVIEWS

Heredity in Ophthalmology. By JULES FRANCOIS. 731 pp., $23.00. The C. V. Mosby Co., St. Louis, Mo., 1961.

Heredity in Ophthalmology is a 700-page book which represents a vast labor and an admirable capacity for organization. The book has real merit and undoubtedly will be extremely valuable to all diagnosticians, particularly ophthalmologists. The material is so arranged that the application of basic principles of genetics is clarified for the novices in that science, and we are many.

The book contains forty-three chapters and is divided into four parts. Part I contains twelve chapters concerned with the basic principles of genetics as applied generally and a chapter outlining applications of biostatistics. Part II deals with genetics as applied to ophthamology. Part III concerns hereditary diseases of the eye on the basis of topical diagnosis. Part IV covers general diseases and ocular manifestations largely from the standpoint of heredity. In this section a variety of syndromes are described with particular attention paid to the hereditary features.

An outstanding merit is the bibliography made available at the end of each section. The descriptions are clear, not overly long, the illustrations are excellent and numerous and the printing is admirable. The index seems adequate and in any event the arrangement makes it easy to find what the book offers on most ophthalmological problems.

FRANK B. WALSH

Management of Obstetric Difficulties, 6th edition. By J. ROBERT WILLSON. 687 pp., $16.50. The C. V. Mosby Co., St. Louis, Mo., 1961.

This volume serves very nicely the purpose of a practical work on the management of various obstetric difficulties. It starts, perhaps logically enough, with infertility and leads on through its successful treatment to the subsequent complications thereof. The contents are presented simply and factually without overburdening by historical or theoretical discussion. Current treatments and drugs are handled in a similar fashion, making this edition a useful reference for both house staff training and a busy practice.

TIFFANY J. WILLIAMS

Atlas of Obstetric Technic. By J. Robert Willson. 304 pp., de luxe edition $14.50. The C. V. Mosby Co., St. Louis, Mo., 1961.

This book hues close to its title as an atlas of obstetrical operations. It covers systematically all the common and some not so common procedures in a straightforward step-by-step manner. Many obstetricians may differ with minor points here and there but the major principles follow widely accepted techniques.

There is a minimum of text limited to a few summarizing paragraphs or page or so introducing each procedure and the explanatory captions for the plates. The text uses a remarkable economy of words but withal has a notable clarity of meaning. This leads to oversimplification at times.

Most of the illustrations are simple ink line drawings and are generally clear but are utilitarian rather than artistically inspired. Oblique views are used in some places where direct frontal views would have been clearer. The cesarean section illustrations are less satisfactory than some available elsewhere.

The material in this book will appeal chiefly to the student and the house officer beginning his training. For this reason a less expensive format would have been desirable.

Eleanor Delfs

Allergie und Allergische Erkrankungen. By E. Rajka. Vol. I, 638 pp., $15.00. Vol. II, 1003 pp., $18.00. Kultura Publications Dept. Budapest, Hungary, 1959.

The term "allergy" as originally coined by von Pirquet designated a fairly narrow field of symptoms, syndromes, or diseases, characterized by altered sensitivity of an organism. Experimental and clinical observation have expanded the original concept to such an extent that hardly any section of medical theory or practice has failed to be influenced. Any attempt to present the accumulated knowledge in approximately complete form would now be beyond the reach of a single author. Even the editorship of such work is a formidable undertaking. Dr. E. Rajka of Budapest, Hungary, who for many years has been active in the study of allergic phenomena especially in his field of dermatology, has assembled a team of twenty-eight contributors, all of Hungary, and he has himself written many major sections of this two-volume publication. His confession in the introductory remarks that the inclusion of so many data and opinions may have gone a little too far, disarms any critical attack on this account. It is indeed difficult to sift really significant contributions to our knowledge from trival or inaccurate observations. Furthermore, when so many authors collaborate, repetitions and contradictions are to some degree inevitable. However, elimination of some of the more obvious repetitions, and omission of doubtful, unsubstantiated, or purely speculative material would have increased the value of the work.

The term allergy is applied in the sense of the unitarian theory which holds that all the different kinds of antibodies whether demonstrated *in vivo* or *in vitro*, are an essential part of allergic processes and that allergy and immunology should be part of the science of "allergology". Rajka emphasizes that antigen-antibody reactions can take place at any time, whereas such additional factors as allergic "diathesis", heredity and dysfunction in the vegetative system, are necessary to produce allergic diseases. The first volume of over 600 pages is devoted entirely to discussions of general problems and of methods. There are fourteen chapters, dealing with classification, the various factors of the antigen-antibody reaction including origin and transportation of the antigen and the antibody, experimental anaphylaxis, modes of sensitization, pathological anatomy of allergic diseases, and general principles of diagnosis and treatment. There

is also an excellent chapter on immunochemistry of the antigens and antibodies, and finally a section on serology with detailed description and many illustrations of such modern methods as geldiffusion, immunoelectrophoresis, hemagglutination, preparation of virus antigens and virus antibodies.

The second volume of the work, containing over 1000 pages, presents discussions of allergic diseases of the respiratory tract, intestinal tract, of the blood and blood-forming organs, of the kidneys, of the nervous system, of eye and ear, of the male and female genital tract, and of the skin. Allergic factors in collagenous disease, in rheumatic fever, in acute and chronic infectious diseases with special consideration of tuberculosis and syphilis are thoroughly presented.

With a work of this kind it is impossible to do more than select a few points for critical consideration. Whereas the chapters dealing with allergic manifestations on the skin extend to over 300 pages and contain a wealth of instructive illustrations, it is surprising to find that less than 100 pages are devoted to allergies of the respiratory tract, and that no illustrations of hayfever plants and their pollens are included. Those who expect to find a discussion of details of treatment of the various allergic conditions will be disappointed. Only short remarks are devoted to therapy, and they are mostly in small print as an appendix at the end of the various chapters. In the treatment of allergic rhinitis and bronchial asthma the use of autogenous vaccine is believed to be superior to extracts from environment allergens, but no evidence is offered in support of this dictum. Methods of preparing extracts for skin testing and treatment are not discussed. To omit skin tests with the pollens if bronchial asthma is perennial, and to disregard feathers and house dust, if symptoms are more prominent in the daytime or outside as advised on page 480, Volume I, is bound to produce incomplete results of treatment. More generally, one might disagree with some classifications and opinions, or with the failure to take a definite stand to some problems. However, much of our knowledge is in a fluid stage, and this explains the frequency with which well founded theories and opinions are immediately followed by experimental work and theories which lead to directly opposite conclusions. There is no attempt to come to final decisions and to give guidance for the uninformed reader. In the trend common to publications in Central Europe all probabilities and possibilities, no matter how remote, have been given ample space. Consequently, the work is valuable not to the student or beginner, but as a rich source of references for the research worker and the experienced clinician. Each of the many chapters of the two volumes is followed by extensive bibliographies, containing up to 500 titles. The literature of the western European and American countries has been assiduously studied and digested. References to valuable work done and published in Hungary will be of interest to many readers. A cross index of authors and a separate subject index covering together 114 pages conclude the remarkable work.

It should be added that the present text is a German translation of the original Hungarian. This translation has been executed and printed in Hungary. It reads very smoothly in most parts, but has some Austro-German idiomatic terms and sentence constructions which might puzzle even the reader well familiar with the German language. Paper, print, and the more than 300 illustrations are of outstanding quality. The editor and his collaborators are to be congratulated for an extraordinary accomplishment in a still ill defined but increasingly important field of medicine.

S. Walter Landau

Surgery for Nurses, 7th edition. By JAMES MORONEY. With a Foreword by Dorothy M. Smith. 792 pp., $7.50. E. & S. Livingstone, Ltd., Edinburgh & London, 1961. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

This, the seventh edition of the book, is a well written and beautifully illustrated text which is, on the whole, encyclopedic in nature. The surgical disorders are illustrated, nursing procedures are carefully spelled out, and the equipment to be used is shown.

It is of interest that there are differences between the British and American methods of handling certain diseases. Very little is said in this book about the large, radical, tumor operations commonly carried out in the United States and there is little or no mention of cardiovascular surgery. It is also of interest to note the author's attitude toward the surgical therapy of esophageal hiatus hernia, which is somewhat less than enthusiastic.

EDWARD S. STAFFORD

Progress in the Treatment of Fractures and Dislocations 1950–1960. By THOMAS B. QUIGLEY and HENRY BANKS. 102 pp., $2.50. W. B. Saunders Co., Philadelphia, Pa., 1960.

The publication of this progress report as a small book was eagerly anticipated by orthopaedic surgeons who read it when it first appeared in serial form in the New England Journal of Medicine in 1960. The authors have summarized from the literature trends in the treatment of each general type of fracture and dislocation over the last ten years. This feat has been accomplished with commendable brevity in the text and with a sufficiently large and well chosen bibliography that it will serve as a handy source book. Many of the trends are presented in the light of the authors' experiences with similar or different therapeutic techniques used primarily at the Peter Bent Brigham Hospital. There are no illustrations, graphs or tables. The book does not describe in fine detail how to perform any manipulation or operation. It will be less valuable to the medical student, junior house officer, or inexperienced general practitioner confronted with a fresh fracture than a new edition of almost any of the large available textbooks on this subject. It will be of considerable interest and value, however, to those who are already familiar with accepted techniques of fracture treatment and are anxious to refresh themselves on who has recently modified these techniques and how and why.

GERHARD SCHMEISSER, JR.

Protein and Amino Acid Requirements in Early Life. By L. EMMETT HOLT, JR., PAUL GYÖRGY, ERWARD L. PRATT, SELMA E. SNYDERMAN and WILLIAM M. WALLACE. 63 pp., $1.00. New York University Press, New York, N. Y., 1960.

This small monograph reexamines protein and amino acid requirements of the growing child in the light of some of the more recent work. In part the data presented are based on studies conducted by Dr. Holt and his group at Bellevue Hospital on the amino acid requirements of small infants.

The book, although providing a complete guidepost to both the recent and classical literature in this field, remains extremely practical at all times. For example the question of the minimal adequate protein intake of premature infants is considered. The authors suggest that maximal nitrogen retention is not necessarily a criterion for an adequate protein intake, and propose that a diet with as little protein as 2 gm./kg./day may well be adequate for optimum growth in the vast majority of premature infants.

The authors also discuss protein requirements in disease and for optimum im-

munity. The possible adverse effects of high protein intake are brought up. More recent work on the pathophysiology of kwashiorkor is reviewed.

In the last section of the book, dealing with social aspects of protein nutrition, one is struck by the correlation between high protein intake and arteriosclerosis. This once again confirms the suspicion that the latter may well be the result of improper pediatric dietary practices.

In short, this is a book which is heartily recommended to pediatricians, nutritionists, and persons in related fields.

JOHN H. MENKES

Pediatric Nursing, 4th edition. By GLADYS BENZ. 572 pp., $6.00. The C. V. Mosby Co., St. Louis, Mo., 1960.

Although this book was written primarily for student nurses as a guide for gaining a basic understanding of child care, because of its scope it should be of interest to all those concerned with the welfare of children. The focus throughout is on understanding the individual child, his place in the family and the community.

By emphasis on the basic principles of child growth and development and of mental health and by showing how they can be applied in specific instances the author has provided a broad background of information for understanding the child. The nursing care of the child with abnormal conditions and diseases is covered in a large section of the book. The last chapters are devoted to changing attitudes toward child care and the development of today's approach to his welfare. Included is a brief description of many community problems and services.

MARY KOPENHAVER

BOOKS RECEIVED FOR REVIEW

Advances in Thyroid Research. Transactions of the Fourth International Goitre Conference. General editor, ROSALIND PITT-RIVERS. 537 pp., $20.00. Pergamon Press, Oxford, England, 1961.

Atlas of Human Anatomy, revised 6th edition, 1961. Edited by M. F. ASHLEY MONTAGU and ERNEST F. KERBY. 180 pp., $2.95. Barnes & Noble, New York, N. Y.

British Medical Bulletin, Vol. 17, No. 2, May, 1961. Foetal and Neonatal Physiology. Scientific editor, K. W. CROSS. Introduction by R. A. McCANCE. 176 pp., $3.25. Published by the Medical Department, The British Council, London, England.

Clinical Orthopaedics, No. 19. Soft-Tissue Tumors. Edited by ANTHONY F. DE PALMA. 307 pp., $7.50 single copy. J. B. Lippincott Co., Philadelphia, Pa., 1961.

Essential Pathology. By ROGER D. BAKER. 638 pp., $9.50. The Williams & Wilkins Co., Baltimore, Md., 1961.

Essential Urology, 4th edition. By FLETCHER H. COLBY. 603 pp., $8.00. The Williams & Wilkins Co., Baltimore, Md., 1961.

Glaucoma. Transactions of the Fifth Conference, March, 1960. Edited by FRANK W. NEWELL. 329 pp., $9.00. The Josiah Macy, Jr. Foundation, New York, N. Y., 1961.

Histology, 4th edition. By ARTHUR WORTH HAM and THOMAS S. LEESON. 942 pp., $11.00. J. B. Lippincott Co., Philadelphia, Pa., 1961.

Hypertension. Chemical and Hormonal Factors. Vol. IX, Proceedings of the Council for High Blood Pressure Research, American Heart Association, November, 1960.

Edited by FLOYD R. SKELTON. 806 pp., $2.50. Published by the American Heart Association, Inc., New York, N. Y., 1961.

Inflammation and Diseases of Connective Tissue. A Hahnemann Symposium. Edited by LEWIS C. MILLS and JOHN H. MOYER. 900 pp., $16.00. W. B. Saunders Co., Philadelphia, Pa., 1961.

Medical Pharmacology. Principles and Concepts. By ANDRES GOTH. 551 pp., $11.00. The C. V. Mosby Co., St. Louis, Mo., 1961.

Microbial Reaction to Environment. The Eleventh Symposium of The Society for General Microbiology. Edited by G. G. MEYNELL and H. GOODER. 416 pp., $7.50. Cambridge University Press, New York, N. Y., 1961.

Oakes Dictionary for Nurses, 11th edition. By NANCY ROPER. 492 pp., $2.75. E. & S. Livingstone, London, 1961. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

FINE STRUCTURE OF THE SQUID RETINA[1]

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The Johns Hopkins University School of Medicine and the Marine Biological Laboratory, Woods Hole, Massachusetts

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INTRODUCTION

The fine structure of the squid retina has many features of particular interest to the anatomist and physiologist investigating photoreception. Phylogenetically, the cephalopods have evolved an elaborate dioptrical apparatus comparable to the vertebrate eye yet derived by a different embryologic process. The cephalopod eye forms as an ectodermal invagination of the head region while the vertebrate eye forms as an ectodermal evagination of the forebrain. This developmental sequence results in two morphologically distinct retinae: the direct retina of the cephalopod in which the incident light impinges upon the photosensitive surface initially, and the indirect retina of the vertebrate in which light passes through the neural layers before reaching the receptor surface.

Studies of cephalopod retinae in the latter half of the nineteeth century demonstrated the basic structural organization of these receptors (1, 2, 3, 4). A single layer of polarized retinula cells was shown oriented parallel to the incident light. The apical portion of these cells was thought to contain the photosensitive pigment while the basal portion formed the primary afferent nerve fiber. Although the relationship between the outer segments of the squid were known to differ from other cephalopods, difficulties in preparatory techniques greatly limited any detailed analysis.

The outer segments of several cephalopods have been incompletely studied with the electron microscope (5, 6). Micrographs of these segments have shown ordered structures of dense lamellae, a pattern found in all photoreceptors (7). In the vertebrate eye the lamellae represent "intucking folds of the plasma membrane" (8) while in invertebrates larger, tubular extensions from the cell membrane form the receptor surface. The previous studies (5, 6) suggested that the cephalopod eye exhibits a lamellar structure analogous to other invertebrates.

The present study confirms the basic structure elucidated by the light

[1] This investigation has been aided in part by Grants B-2173 and B-2182 from the National Institutes of Neurological Diseases and Blindness, U.S. Public Health Service to Dr. A. J. de Lorenzo and Grants from the Josiah Macy Foundation to the author during elective research quarters in the School of Medicine curriculum.

[2] Henry Strong Denison Scholar 1961–1962.

microscopists. It further shows that the lamellae consist of tubular, microvillous extensions from the cell membrane and that the structural relationship of the outer segments differs from that described for other cephalopods. A complex lamellar system deriving from the endoplasmic reticulum (myeloid body) has been found in the inner segment of the retinula cells.

METHODS AND MATERIALS

Fresh squid (Loligo pealii) were obtained at the Marine Biological Laboratory at Woods Hole and were fixed with 2 per cent osmium tetroxide, in veronal acetate buffer, at 4°C. for periods ranging from one to three hours. Other eyes were fixed in 5 per cent potassium permanganate for two to four hours. These fixatives were either injected into the vitrous cavity or 1 millimeter pieces of the excised eye were immediately placed in the cold fluid. All fixation was performed in the dark. After dehydration in ethanol or acetone, the specimens were infiltrated and embedded in araldite. The sections were cut with a Porter-Blum microtome utilizing glass knives and mounted on grids covered with thin carbon films. An RCA EMU 3C Electron Microscope was used in this study. For light microscopy, the eyes were fixed in glacial acetic-formol-ethanol mixtures and Susa's fixative; embedded in paraffin and stained with phosphotungstate, hematoxylin and eosin, toluidine blue, and Protargol.

RESULTS

A. General Appearance in the Light Microscope

The retina, consisting of radially oriented retinula cells, appears as a four layered structure in the light microscope (Fig. 1). The following arbitrary divisions are useful for descriptive purposes.

(*a*) *Outer Segments*

The outer segments are portions of the retinula cells containing the photosensitive pigment and whose plasma membranes form lamellar structures. These structures are oriented so that they are the region first of the retina to receive the incident light.

(*b*) *Rod Base*

A dense layer of pigment is concentrated between the inner and outer segments. A row of nuclei can be distinguished directly below this pigment. These epithelial cells are continuous with the band of pigment epithelium at the edge of the retina and rest upon a basement membrane in the rod base region.

(*c*) *Inner Segments*

Beneath the pigment and the epithelial cell nuclei are the retinula cell bodies with their large nuclei. The cytoplasm of these cells contains the "myeloid bodies" which are seen in osmiumtetroxide fixed preparations.

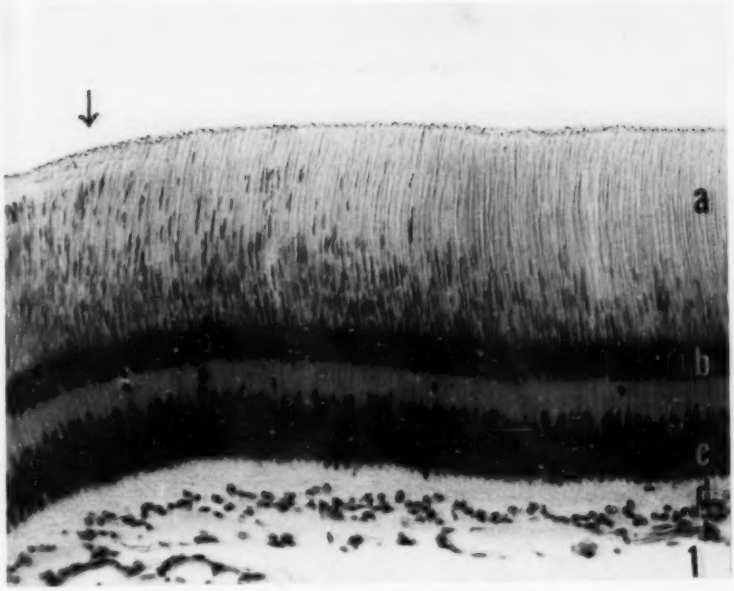

FIG. 1. This light micrograph is a longitudinal section through the entire retina. Pigment can be seen at different levels in the outer segments (a). At the base of the outer segments a dense pigmented layer (b) is visible with the "epithelial" cell nuclei directly below. The dense nucleated region in the inner segment contains the cell bodies (c) of the retinula cells. The plexiform region (d) is unstained by hematoxylin and eosin. The direction of the incident light is represented by the arrow. Approx. ×200.

FIG. 2. This light micrograph is an oblique section through the basal portion of the retina stained by Bodian's Protargol method. This region illustrates the formation of an optic nerve fiber bundle (op.n.b.). Two distinct fiber diameters are visible. The meshwork of the plexiform region (pl) is seen above as well as the nuclei of the inner segments (i.s.). Approx. ×200.

(*d*) *Plexiform Layer*

Directly below the inner segments is a distinct nerve fiber layer, shown in Figure 2, a Protargol stained section. Another row of cells forms the base for this plexiform region and separates it from bundles of optic nerve fibers and blood vessels.

B. Appearance in the Electron Microscope

Examination of the retina with the electron microscope confirms the structural organization seen with the light microscope. For orientation purposes the observations will be discussed in terms of the four layers described above.

Measurements from electron micrographs show the cross sectional width of the retina to be approximately 0.5 mm. The ratio of the length of the outer segments to the length of the inner (excluding the plexiform region) is about 2.1 to 1.

Outer Segments

The outer segments are projections of the retinula cell body. The length of these segments, measured between the rod base and the internal limiting membrane, is 0.3 mm. throughout the retina, diminishing only at the retinal margins.

The tips of the segments are separated from the vitrous cavity by the internal limiting membrane (the "limitans" of Grenacher (2) and the "homogene membrane" of Schultze (4). This limiting membrane has no apparent fine structure, even with the additional resolving power of the electron microscope. It appears as an amorphous structure 5 microns thick (Fig. 3). On the retinal side of this membrane the "limitans fibers" (Grenacher) are seen between the rod tips (Fig. 3). Grenacher claimed that these fibers were derived (in octopus) from processes of the epithelial cells found at the rod base region. Our observations as well as those of von Lenhossek have not demonstrated this continuity. Von Lenhossek postulated that these fibers resulted from shrinkage artifacts (9). Electron micrographs, however, reveal well defined and constant processes that appear to derive from the retinula cells and have canalicular profiles containing dense osmiophilic granules (Fig. 3).

The plasma membrane of the outer segment forms lamellated, microvillous projections perpendicular to the light patch (Figs. 4 and 5). In longitudinal sections the microvilli from one cell are seen to abut against the microvilli from an adjacent cell. Electron micrographs have shown that the length of these microvilli are uniform in individual retinae but variation in the conditions of fixation will produce slight changes in the dimensions. The average length of a microvillus is 1.1 microns and the

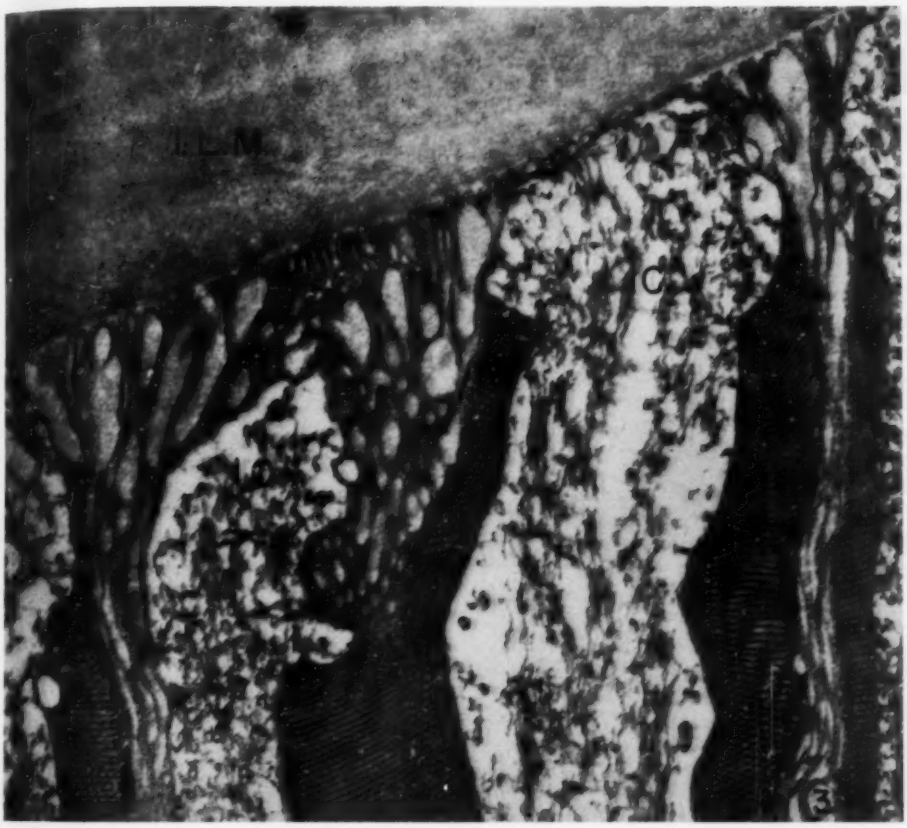

FIG. 3. This is a low power electron micrograph through the region of the rod tips. The internal limiting membrane (i.l.m.) is seen as an amorphous structure 5 to 6 microns thick. The tips of the outer segments do not form the microvillus extensions typical of the remainder of the outer segment but rather "finger-like" projections which apparently play a rôle in the attachment of the internal limiting membrane. The intracellular compartment (IC) is labeled. ×10,000.

average diameter is 0.1 microns. The microvilli from a retinula cell cut in cross section appear as regular hexagonal arrays, i.e., any single microvillus is surrounded by six others (Fig. 6). In some longitudinal sections showing the length of the microvilli, the base of each villus is constricted to one-half its largest diameter yet remains continuous with the plasma membrane of the cell (Figs. 5 and 7). When a thin section (200 to 400 Å) does not pass through this constricted base, the villus appears disk shaped and the continuity with the plasma cell membrane is not visible.

When tangential sections of the retina are examined the structural

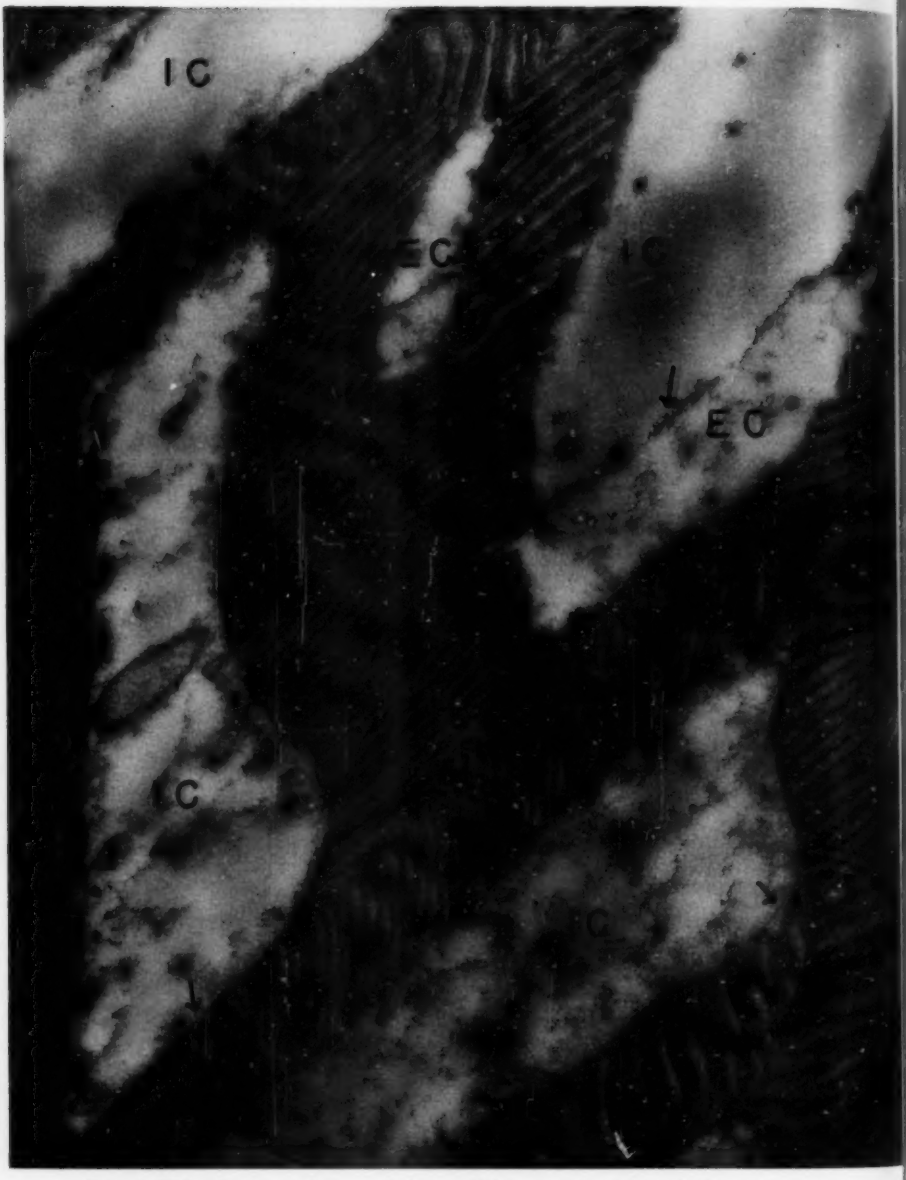

FIG. 4. This electron micrograph is an oblique section through the rod outer segments. The intracellular (Ic) and extracellular (ec) compartments are labeled. The arrows point to that portion of the plasma membrane which has not formed the microvillus extensions. This portion of the membrane usually abuts against the microvillus extensions of the neighboring retinula cell outer segment. Permanganate fixation. ×22,500.

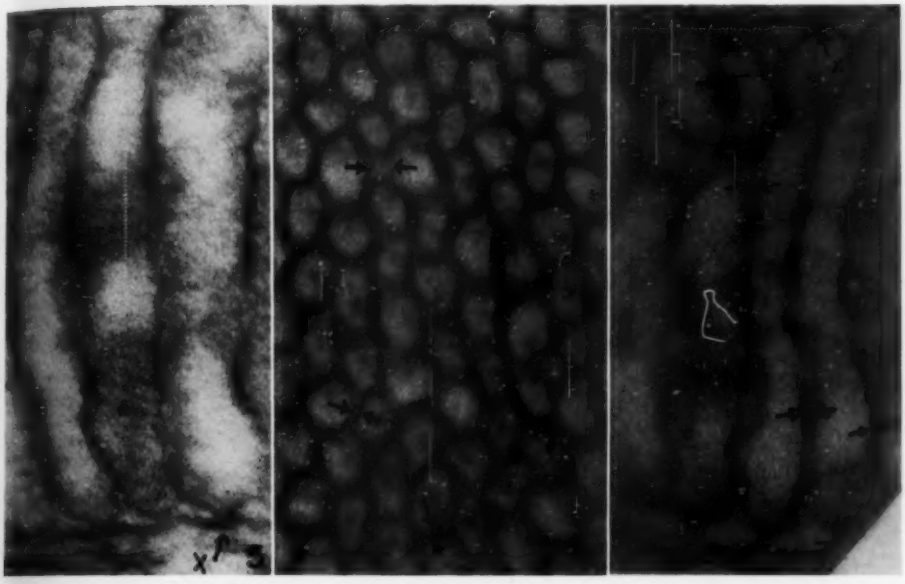

FIG. 5. This high power electron micrograph shows the fine structure of several microvillus extensions. The arrows (x) point to the base of the extensions where they can be seen as continuous with the plasma membrane and arising as evaginations of this membrane. It is also evident that this base region is much constricted relative to the rest of the extension. Permanganate fixation. ×112,500.

FIG. 6. This high power electron micrograph is a cross section through the microvillus extensions. They are seen in hexagonal array. The paired arrows point to regions between which extracellular space is visible. In the intracellular region of each microvillus a dense osmiophilic region is seen. Permanganate fixation. ×80,000.

FIG. 7. This high power electron micrograph is similar to Figure 5. This illustrates, however, that each microvillus extension does in fact have a distinct membrane. The paired arrows point to the regions where this is evident. The unpaired arrow (x) is the region of the microvillus base (opposite orientation to Fig. 5). Permanganate fixation. ×112,500.

pattern becomes more complicated. Instead of the expected cylinder cross section, which would have to appear as circles, we find a complex mosaic (Figs. 8 and 9 insert). The analysis of this mosaic must begin with identification of the cell membranes. These are best seen in sections that have become teased apart during fixation. While most of the plasma membrane forms microvilli, small areas of the membrane remain unspecialized (Fig. 4). It is this portion of the membrane (without microvilli) that is usually found in proximity to the microvilli of an adjacent outer segment. When this is the case, only the highest resolution will distinguish the two mem-

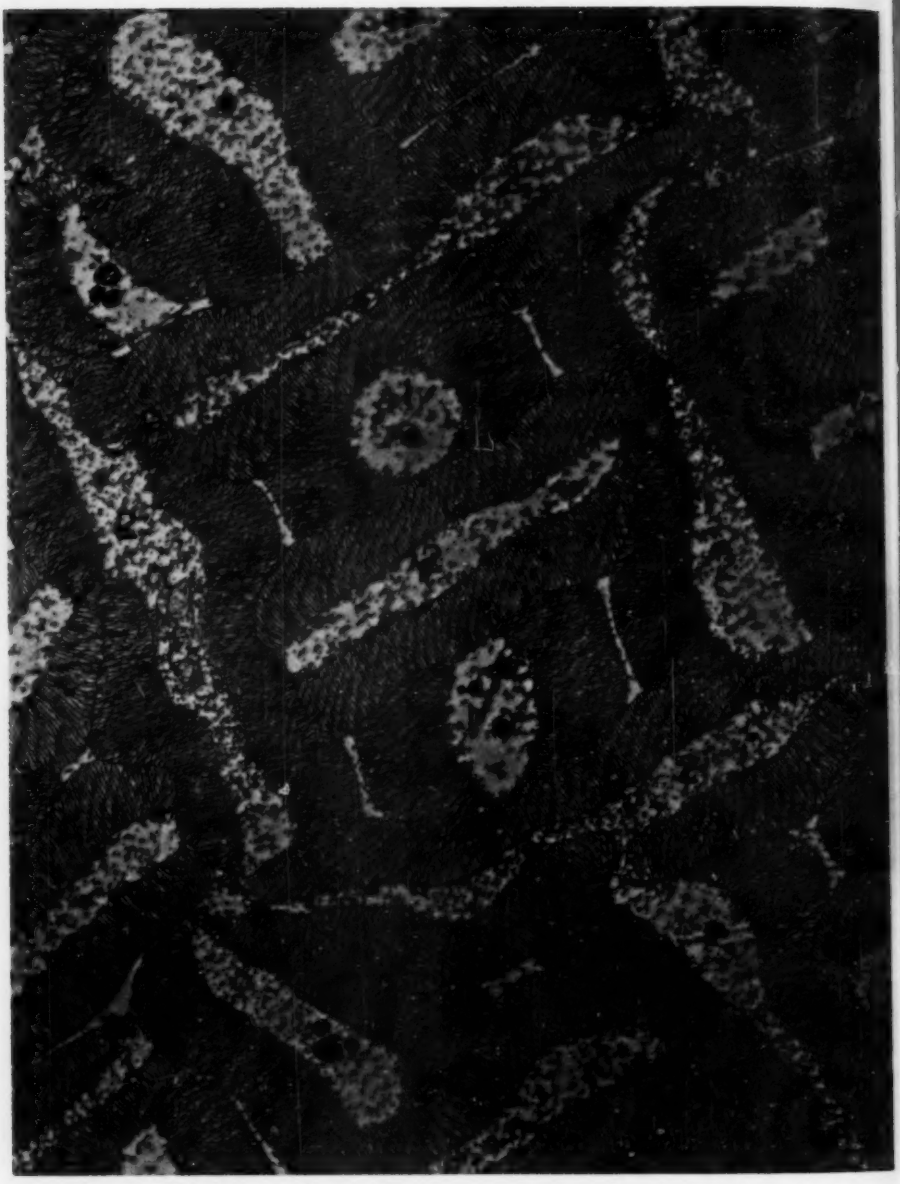

FIG. 8. This electron micrograph represents a cross section through the rod outer segment illustrating the common organizational pattern. The two rod types are labeled 1 and 2. Rod type 1 contributes to two rhabdomes and rod type 2 contributes to four (see text). The extracellular space is seen to be quite small relative to the intracellular. In the cytoplasm of these outer segments the dense staining bodies are pigment granules. Permanganate fixation. ×3800.

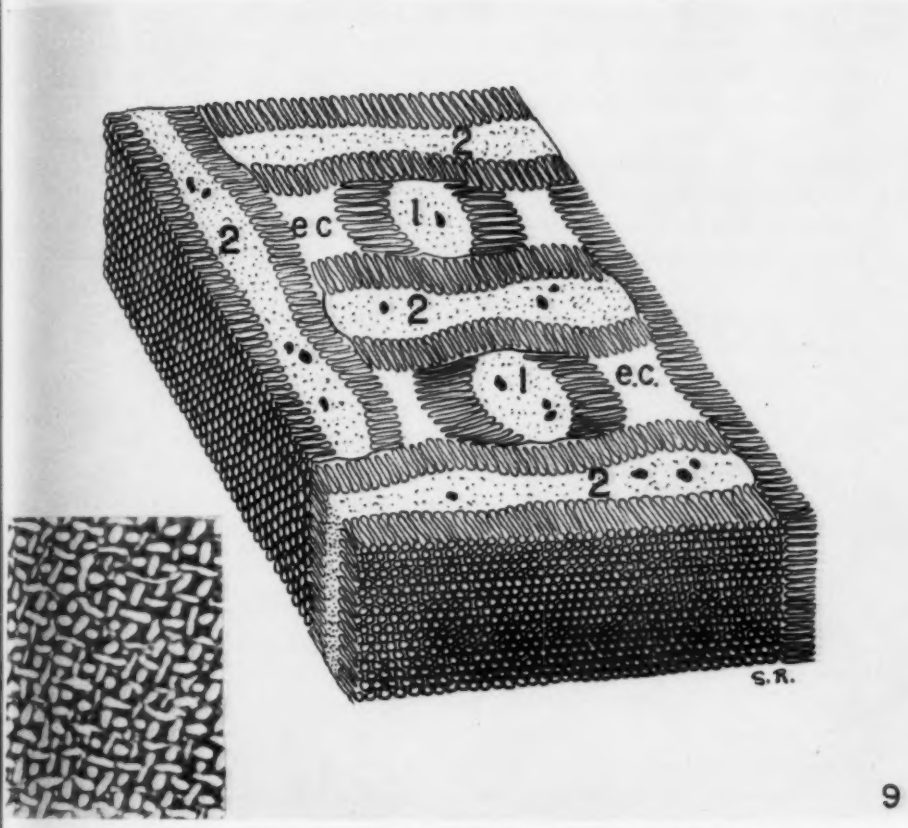

FIG. 9. This drawing is a schematic three dimensional reconstruction of the rod outer segments. This structure has been based on that seen in Figure 8. The extracellular space (ec) has been exaggerated for clarity. The rod types are labelled 1 and 2. The microvilli are also seen to form a hexagonal array in cross section. The insert is a low power light micrograph illustrating the irregular lattice and perpendicular orientation of the type 2 segments.

branes. The boundaries of the intracellular and extracellular spaces are also distinguishable in the teased preparations.

Once the cell boundaries have been determined it becomes possible to analyze a section such as Figure 8. A three dimensional construction of Figure 8 is shown schematically in Figure 9 and represents a composite of many retinas examined. At least two types of retinula cell outer segments are evident and are labeled Type 1 and Type 2. These are circular (labeled 1) and elliptical (labeled 2) in outline. Light micrographs (Fig. 9 insert) reveal no regular lattice structure as found in other cephalopods.

The pattern most frequently observed is illustrated in Figure 8 where each type 1 segment is found surrounded by four type 2 segments. In a count of 1000 cells from different sections 690 were type 2 and 310 were type 1; a few transitional forms are also present.

There are constant directional relationships between the outer segments. The elliptically outline segments (labeled 2) are perpendicularly oriented to each other (Figs. 8, 9). In the electron micrographs this perpendicular orientation is seen in the microvilli of both type 1 and 2 segments. The outer segments that are contiguous have their microvilli at 90 degrees to each other, while the segments whose microvilli are separated by extracellular space are parallel (Fig. 8). The microvilli from a single outer segment are found in two parallel rows on opposite sides of the cell. Whether these arrangements are functionally significant remains to be seen.

The number of outer segments per square centimeter is 5–6 x 10^4 in osmium fixed preparations. The total surface areas of a single outer segment will be related to the number of microvilli per segment. This number is not constant as two segment types are present. Counts of the number of microvilli per cell show that type 1 segments have approximately 200,000 and type 2 have approximately 700,000 per cell.

Rod Base Region

The rod base region is defined as the area from the rod outer segment to the basal portion of the "undifferentiated epithelial" (von Lenhossek) or "limitans" (Grenacher) cells. The rod base corresponds to the portion of the vertebrate eye which appears as a cilium in cross section. This region is 32 microns thick and is characterized by a dense accumulation of pigment granules. Analysis of this area with the light microscope requires agents such as nitric acid to dissolve the pigment. Electron microscopy makes such treatment unnecessary and shows the constricted portion of the retinula cell between the epithelial cells (Fig. 10). The cytoplasm of the retinula cell at this level contains a dense collection of pigment granules. These granules are circular and elliptical in outline; usually oriented with the long axis parallel to the longitudinal axis of the cell. In the potassium permanganate fixed preparations, each pigment granule is surrounded by a membrane (Fig. 11) consistent with the unit membrane structure (10). In osmium tetroxide fixed material, however, this membrane cannot be resolved, perhaps because of the greater overall demonstration of pigment stained with osmium.

Interdigitated between the constricted portions of the retinula cells are the "undifferentiated epithelial cells" of von Lenhossek (Fig. 10). The epithelial cells, confined to the rod base region of the retina, contain large ovoid nuclei located at the basal portion of the cells. The cytoplasm con-

FIG. 10. This electron micrograph is an oblique section through the rod base region showing the epithelial cells and surrounding retinula cells. The retinula cells (RC) contain a high concentration of pigment granules (each surrounded by a membrane) and the myeloid figures (mb). The mitochondria can be seen close to the plasma membrane of the retinula cells. The epithelial cells (ec) are less densely stained with large nuclei (nuc) and no nucleoli or myeloid figures. Permanganate fixation. ×4800.

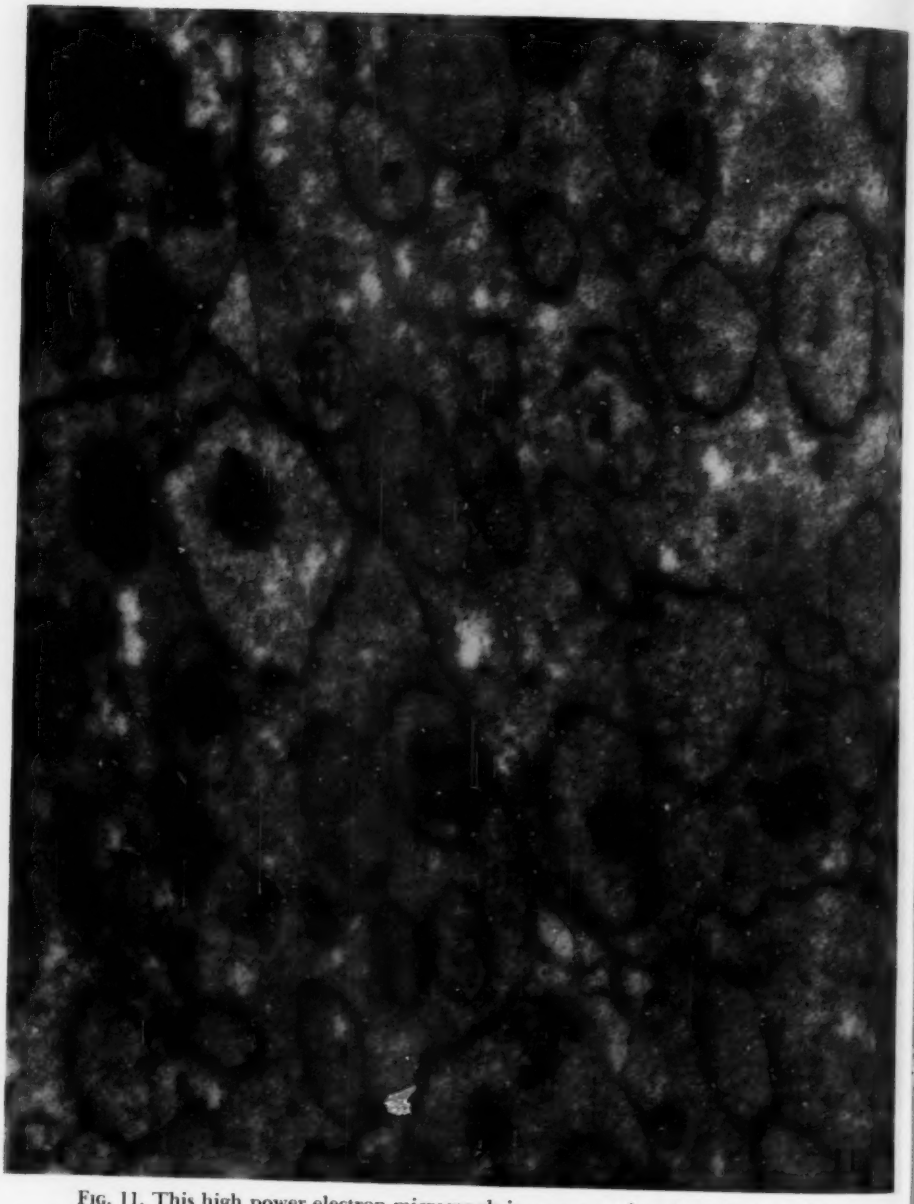

FIG. 11. This high power electron micrograph is a cross section through the rod base region illustrating the fine structure of the pigment granules. Each pigment granule is seen surrounded by a membrane. The pigment, *per se*, shows no fine structure. Permanganate fixation. ×13,250.

tains mitochondria and pigment granules of identical structure as those found in the retinula cells. The pigment is polarized, i.e., it is found only in the apical portion of the cell. These cells can be distinguished from the surrounding retinula cells by: (a) scanty cytoplasm that appears less dense than the retinula cell, (b) fewer pigment granules, (c) absence of cytoplasmic myeloid figures and (d) location only in the rod base region.

Hensen (3) and Grenacher (2) describe a "membrane" at the basal portion of the epithelial cells. Von Lenhossek (9) considered this a "cuticular formation" and a secretion product of the epithelial cell. In our electron micrographs this structure has the amorphous appearance of a basement membrane about 0.2 microns thick, perhaps supporting the interpretation of the epithelial derivation of these cells. Classical studies (2, 3, 9) have suggested that these cells form the internal limiting membrane, since they can be traced to the margins of the retina and are seen there to be continuous with the pigment cells attaching to the lens. In this study the internal limiting membrane has also been traced to the pigment epithelial layer and is found overlying these cells. This membrane was considered by von Lenhossek as a "clotted secretion" of the epithelial cells and comparable to the cupula of the inner ear.

The evidence for pigment migration in the squid has not been well documented, although it has been described in related species (11, 12, 13). Electron and light micrographs have shown pigment at the tips of the outer segments most consistently. Scattered pigment granules have been seen throughout the outer segments, but the changes in pigment location with varying light conditions have not as yet been studied.

Rod Inner Segment

This region extends from the epithelial cell base to the plexiform layer and is about 0.1 mm. thick. The nuclei of the retinula cells are long ovoid structures containing numerous nuclear pores and three to six dense staining "nucleoli" which appear as dense osmiophilic regions in electron micrographs.

A submicroscopic organelle has been observed in the *cytoplasm of the retinula cells* that extends from the rod base region into the portion of the cell that forms the afferent nerve fiber. This structure consists of closely packed, lamellated unit membranes that resemble myelin and are termed *myeloid bodies* (Figs. 10 and 12). It would appear that they are organized as sheets since they exhibit the same appearance in longitudinal and in cross sectional micrographs. These are unlike similar units found in the frog retina (14) in that these structures are found in the *receptor cells* and *not the pigment epithelium*. In the basilar portion of the retinula cells these figures can be seen dispersing, becoming indistinguishable and/or

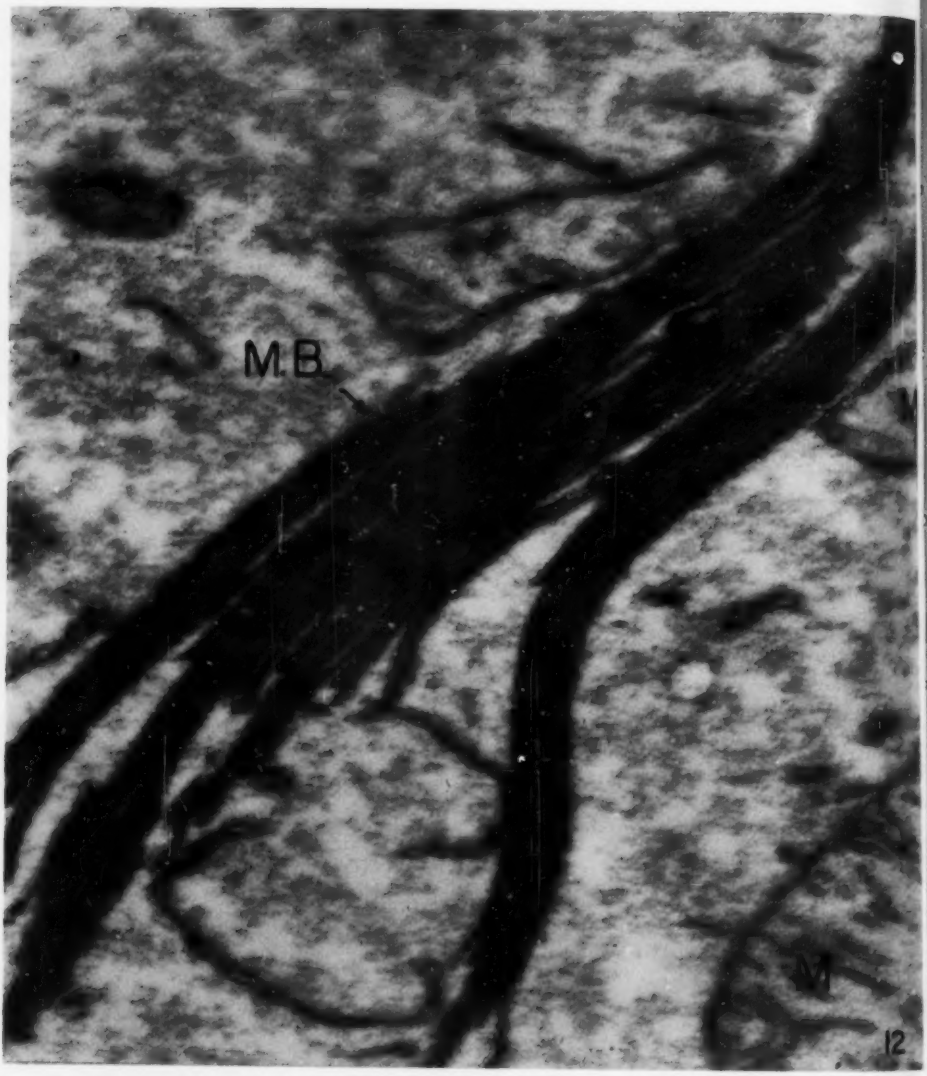

FIG. 12. Electron micrograph illustrating the myeloid body (MB). This figure was located in the inner segment of the retinula cell immediately below the rod base region. The osmiophilic lines are 100Å apart and are 60–70Å thick. They can further be subdivided into the unit membrane of Robertson with 20–25Å components. (M) mitochondria. Permanganate fixation. ×127,500.

continuous with the endoplasmic reticulum (Fig. 13). The encoplasmic reticulum and the myeloid bodies do not have dense particles attached to or associated with these membranes in either osmium tetroxide or permanganate fixed preparations. The membranes composing the myeloid figures, however, appear more electron dense than the endoplasmic reticulum. Each unit membrane is about 60Å thick and the space between is 100Å.

The vertebrate rod below the modified cilium is highly organized into an "ellipsoid region", i.e., a region containing large numbers of closely packed mitochondria. The squid retina does not show a comparable cluster of mitochondria, though the inner segment above the nucleus contains large numbers of mitochondria. If at all oriented they are in close proximity to the plasma membrane. There is no evidence of a ciliated structure in the receptor cells.

The cells in the inner segment are closely packed in the permanganate fixed preparations and little intercellular space is detectable. On the other hand, in the osmium tetroxide fixed preparations a slightly larger extracellular space is seen. This may be due to the hypotonicity of the permanganate fixative. Thus, volume measurements of cells and extracellular space may well differ depending upon the osmolarity of the fixative used.

In the region of the epithelial cell base the radial orientation of the inner segments is interrupted by a vast capillary network which courses at right angles to the retinula cells. The squid outer segments and rod base regions are avascular as are vertebrate outer segments. A schematic diagram of the retina to the plexiform region is illustrated in Figure 14.

Plexiform Layer

The large number of fine fibers and the plexiform nature of their organization make this layer (38 microns thick) difficult to analyze. Von Lenhossek has provided the most complete and convincing analysis of this region, utilizing the Golgi-Cox technique. He describes two basic retinula cells endings with some transitional forms: (a) Reichenzellentypus and (b) Lumbricustypus (9). According to von Lenhossek, cell type (a) is reminiscent of cells in the olfactory epithelium, i.e., small spindle shaped cells with no side branches. The second type (b) is thicker, with the nucleus often at the basilar pole of the cell and fine, varicose lateral processes. Some of these processes penetrate into the plexiform layer obliquely while others extend horizontally.

Preparations stained with Protargol show this layer as a dense meshwork of interweaving and branching fibers. As the cell bodies do not usually bind the silver stain, it is difficult to follow the interconnections of a

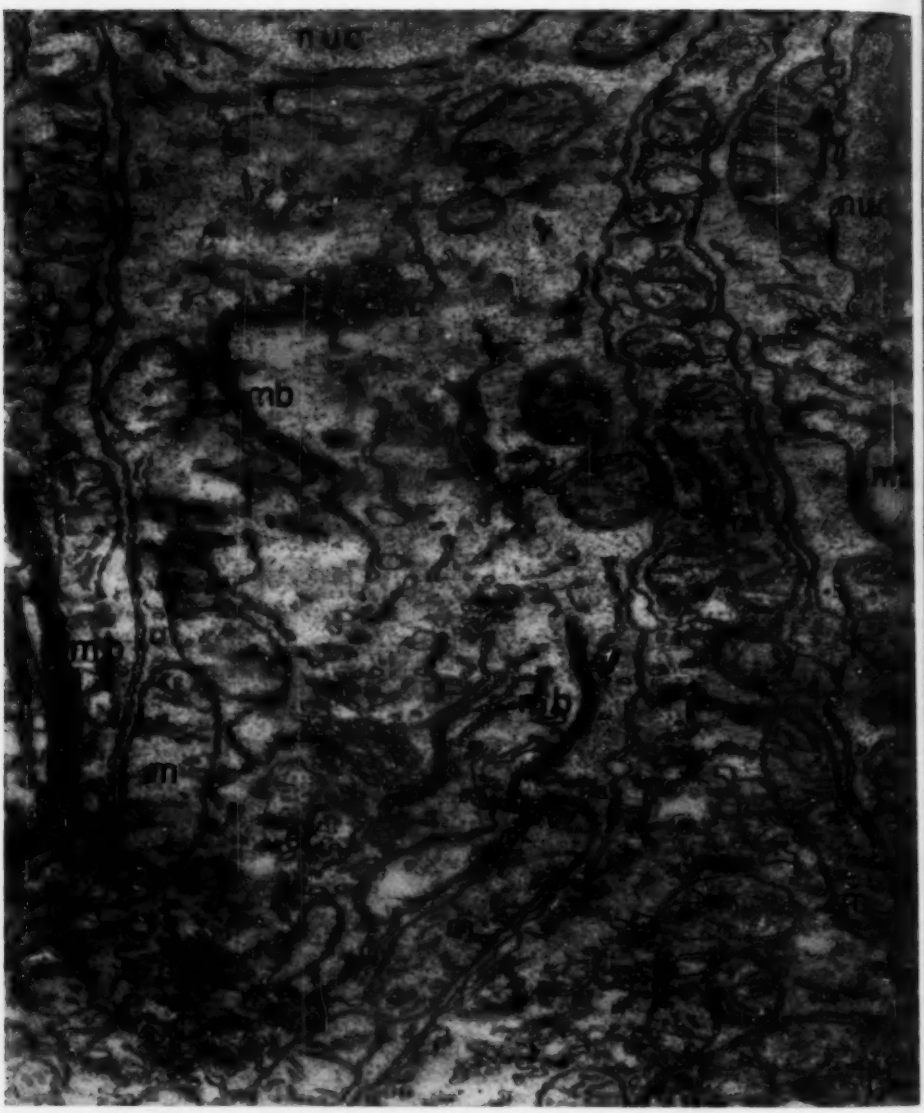

FIG. 13. This electron micrograph shows the lower portions of two reinula cell nuclei (nuc) and the region directly below. Nuclear pores and segments of myeloid figures (mb) are evident and the latter can be seen to become indistinguishable from the endoplasmic reticulum (er). (m) mitochondria. Permanganate fixation. ×23,300.

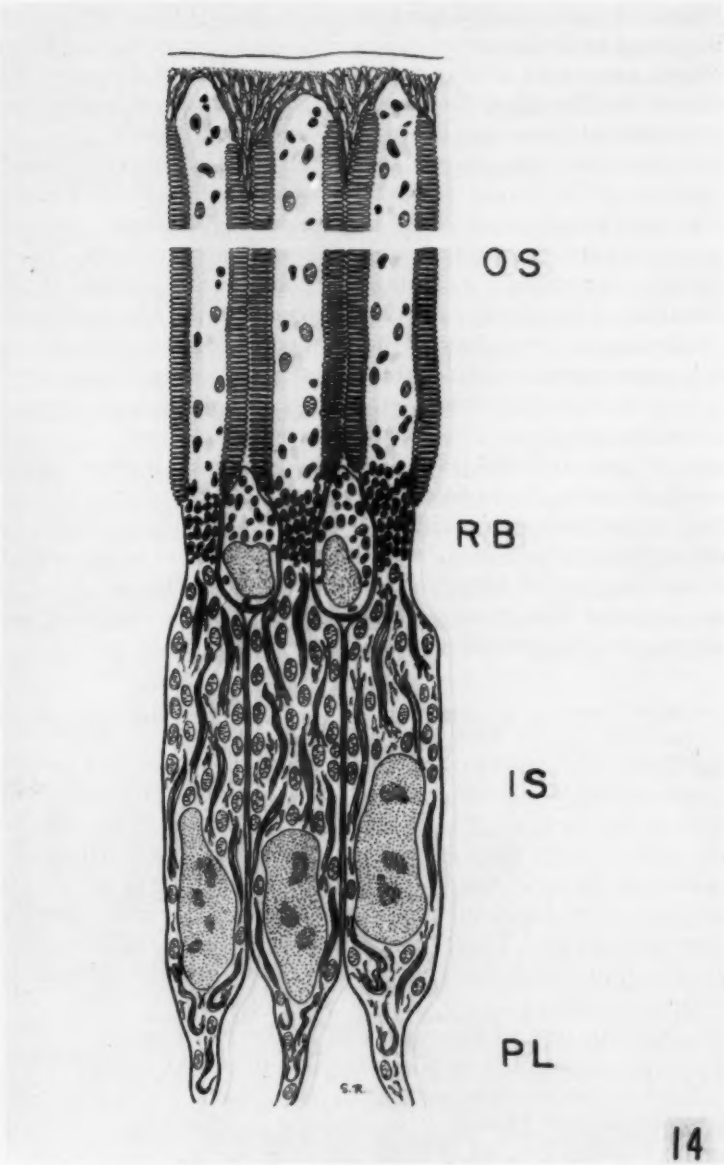

FIG. 14. This drawing is a composite schematic representation of a longitudinal section through the squid retina. The major subdivisions are labeled: OS—outer segment; RB—rod base; IS—inner segment; PL—plexiform layer.

single cell. However, several points are clear. In the optic nerve two groups of fibers may be distinguished by size, i.e., one group consists of numerous fine fibers, the second of less numerous fibers with large diameters (Fig. 2). The large fibers branch as they pass through the plexiform layer and appear to have terminal processes at the bases of the retinula cells.

With the electron microscope, tracing the fibers becomes even more difficult because of the thin sections and the tortuosity of the fibers. The retinula cells are seen to taper at the basal portion of the cell forming the primary afferent nerve fiber (analogous to cell type (a) of von Lenhossek). The tapering zone of the retinula cell contains mitochondria and portions of myeloid figures that are frequently coiled and extend into the fiber process. Other cells whose nuclei are oriented in the basal pole have an appearance suggesting the presence of lateral processes. Processes containing "synaptic vesicles" have been identified in this layer, though it is difficult to determine if these endings are derived from central or peripheral fibers.

A row of large cells lies below the plexiform region in close approximation with the nerve fibers, often appearing to embrace them. The cells resemble the Schwann cells of the fila olfactoria where they ensheath fasicles of unmyelinated fibers (15). Under this cellular layer many optic nerve fiber bundles can be identified surrounded by "Schwann cells" which appear different from those described above. Between the nerve bundles capillaries are seen extending upward into the retinal layer.

DISCUSSION

Cephalopod retinas have been described in terms of rhabdomes and rhabdomeres since Grenacher first suggested this structural organization. While such structures are anatomically distinct and functionally significant in arthropods, the analogous arrangement in squid leads to confusion for several reasons. First, there is no direct evidence of functional significance of such units; secondly, one retinula cell can contribute to portions of two to six rhabdomes, inconsistent with the arthropod structure from which the concept is derived. Therefore, rather than establish arbitrary structural units, the squid retina has been described utilizing the retinula cell as the anatomical unit.

The fine structure of the microvilli in the squid receptors is similar to that of other invertebrate photoreceptors in respect to both origin and size. The functional significance of this lamellar pattern in photoreceptors remains unresolved, though it is tempting to infer that the specialized structure provides a means of fixing the photopigment orientation.

That cephalopod retinas can detect polarized light is suggested by the perpendicular planes of orientation of the microvilli. Moody (16) concluded

that the octopus is capable of polarized light discrimination on the basis of training experiments. De Vries and Kniper (17), on the other hand, consider that the eye detects reflection patterns of light on the environment, patterns which vary with the plane of polarization. Thus, polarization discrimination may be reduced to an intensity factor. The published data of Moody's experiments were not sufficient to determine if this factor was considered.

Pigment migration as a function of light adaptation has not been studied in the squid. Rawitz (12) has shown that the visual rods of *Sepia* are pigmented along their entire length in the light, with a denser accumulation on the vitrous border, at the rod tip. In the dark the pigment is limited to the base of the rod. Pigment migration has also been demonstrated for octopus (13). Whether this is true for squid is currently being investigated.

A membrane surrounds each pigment granule in squid and the myeloid bodies have been seen extending into the region of these granules. Porter and Yamada (14) have described a similar relationship in the pigment epithelium of the frog retina. They suggested that the myeloid body is a light sensitive structure whose membranes are continuous with the membrane of the pigment granules, i.e, a mechanism for pigment migration. This mechanism seems unlikely in the squid as no continuity of the myeloid body and pigment granules has been observed; moreover, the dense pigment layer prevents the incident light from reaching these structures.

In the electron microscope the nerve fiber layer is the area of greatest structural complexity. It is important to determine whether the retinula cell forms the primary afferent axon found in the optic nerve without a synaptic region interposed and if the plexiform layer is a synaptic region with lateral and/or central components. A few points are clear. The bases of the retinula cells taper forming the primary afferent fibers, and portions of the myeloid bodies can be seen extending into the first part of these processes. Further, two distinct fiber diameters are seen in the optic nerve bundles, the larger of the two having branches in the retinal region. Their origin as first or second order afferents or central efferents cannot be determined from these sections, though the branching of the large diameter fibers is compatible with an efferent system. The plexiform layer shows many processes containing vesicles; whether these represent lateral or central inhibitory fibers is again a matter of speculation.

Evidence for centrifugal efferents to peripheral sense organs is controversial. Conclusive evidence has been demonstrated in the mammalian muscle spindle and the crayfish stretch receptor. Suggestive data for centrifugal efferents in the retina has been published in the anatomical

studies of Ramon y Cajal (18), Dogiel (19), and recently by Young (20) in the octopus. The physiological evidence has been presented by Arey (21) in the Gastropod (Planorbus) and by Granit (22) in the curarized cat retina. The squid with its relatively simple organization may provide the experimental situation to test this concept.

SUMMARY AND CONCLUSIONS

1. The squid retina is essentially a unicellular retina. The photosensitive or retinula cells are bipolar sensory cells whose distal segments are highly specialized structurally, that is, the plasma membrane is folded into lamellated microvillous extensions.

2. The outer segments are organized into at least two retinula cell types; the relationship between these units consisting of a complex mosaic different from that observed in other cephalopods. There is also a perpendicular orientation of the microvilli of the retina.

3. The "myeloid body", an organized membraneous component of the cytoplasm is described and considered as a specialization of the endoplasmic reticulum.

4. The fine structure of the pigment granules with surrounding membranes is described and pigment migration is discussed.

5. Nerve fibers are seen in the plexiform region. Processes containing "synaptic vesicles" are present; at least two kinds of nerve fibers are evident and classified on the basis of their overall diameters in stained preparations.

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A DEFICIENCY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN LENS FROM INDIVIDUALS WITH PRIMAQUINE-SENSITIVE ERYTHROCYTES[1]

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A variety of hereditary biochemical changes have been described in the erythrocytes of humans with the primaquine-sensitive type of hemolytic anemia: a low level of reduced glutathione; an abnormal instability of reduced glutathione when red cells are exposed to certain oxidants; increased activity of the enzymes aldolase and glutathione reductase; reduction in activity of glucose-6-phosphate dehydrogenase and catalase; and decreased lipid content of the erythrocyte membrane during a hemolytic episode (1, 2). Although the activity of not one but several enzymes is changed, the primary effect of the mutant gene appears to be an alteration of the function of the enzyme protein, glucose-6-phosphate dehydrogenase. One of the important routes of carbohydrate metabolism in many mammalian cells is the direct oxidation of glucose-6-phosphate by way of the hexose-monophosphate shunt. Since the first step of this pathway is catalyzed by the enzyme glucose-6-phosphate dehydrogenase, an important query is whether the enzyme abnormality of the erythrocyte in primaquine-sensitive persons involves other tissues. Several groups of investigators have already studied this problem, and their observations indicate that the anatomical distribution of the defect varies in different racial groups. The activity of the enzyme in leukocytes and liver from affected American Negroes is normal, or perhaps slightly decreased (3), whereas a significant deficiency has been noted in the leukocytes, platelets, liver, and saliva of non-Ashkenazic Jews (4, 5, 6). Although only a limited number of tissues were studied, the suggestion was made that the enzyme abnormality in American Negroes was limited to the erythrocyte.

The erythron mass and the lens of the eye have certain structural and metabolic similarities: the growth and maturation of cells is associated with the loss of nuclei; the major source of energy is the anaerobic breakdown of glucose; there is a very high concentration of glutathione, and its reduction is partially dependent upon the activity of the hexose-monophosphate shunt; and finally, many drugs and chemicals, e.g. naph-

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thalene, depress the level of glutathione before other toxic effects are observed. On the other hand, there are several significant differences between the lens and the red blood cell. Both originate from a single type of embryonic cell; the lens, however, is ectodermal in origin, whereas the erythrocyte is derived from mesoderm. Furthermore, the mature lens has a single layer of nucleated cells beneath the anterior capsule.

Because of the many similarities between the lens and the erythrocyte, the following studies were conducted to determine if Negroes with a deficiency of red cell glucose-6-phosphate dehydrogenase demonstrated the same abnormality in their lens.

MATERIAL AND METHODS

Source of the Lens

Lens were obtained from two groups of individuals. One group consisted of forty-one Caucasians and thirty Negroes whose lens were removed because of senile cataracts. In each case the lens was extracted by an intracapsular operation, and only those lens with an intact capsule were studied. The other group comprised fifty-eight Caucasians and fifty-nine Negroes whose lens were obtained at the time of autopsy done two to seventy-two hours after death. The removal of the lens was accomplished by sectioning the globe in halves, and the lens with adherent aqueous humor and part of the vitreous body was expressed into a test tube by applying pressure on the cornea. Tubes were stored at a temperature of 5°C. for a period of one to twenty-three hours before doing the enzyme assay. The majority of the lens were grossly clear; a few, however, demonstrated a slight haziness of the capsule.

In those experiments where rabbit lens were used, the method for extracting lens was similar to that applied in the autopsy cases. In some preparations the lens was freed from the zonula by blunt dissection.

Preparation of Lens Homogenate

Most of the aqueous humor and the vitreous body were removed by gently passing the lens over a small piece of Parafilm. The lens was weighed on a Mettler analytical balance, and placed in a 5 ml. Potter-Elvehjem glass tissue grinder. Six parts of chilled 0.14 M sodium chloride were added, and the lens was homogenized with a Teflon pestle. The homogenate was clarified by spinning in a refrigerated centrifuge at about 20,000 × g for 30 minutes. Enzyme assays were carried out on the supernatant.

Enzyme Assays

Activities of glucose-6-phosphate and 6-phosphogluconic dehydrogenase were determined by a modification of the method of Glock and McLean (7). The rate of reduction of triphosphopyridine nucleotide was measured in one cuvette with 6-phosphogluconate as substrate, and in another cuvette with 6-phosphogluconate and glucose-6-phosphate as substrates. Assays were performed in cuvettes with a light path of 1.0 cm. Each cuvette contained 0.1 ml. of lens supernatant, 1.0 ml. Tris buffer, 0.19 M pH 8.0, 0.1 ml. $MgCl_2$, .03 M, 0.2 ml. of .002 M triphosphopyridine nucleotide, 0.1 ml. of .02 M 6-phosphogluconate, and water to a final volume of 3.0 ml. One-tenth ml. of glucose-6-phosphate, .02 M, was added to the second cuvette. The reaction was started

by the addition of triphosphopyridine nucleotide, and the change in optical density was recorded each minute for a period of ten minutes in a Beckman DU spectrophotometer. The value for activity of glucose-6-phosphate dehydrogenase was obtained by subtracting the rate observed in the first cuvette from that of the second. Enzyme values were expressed as the change in optical density per minute per gram of soluble protein. The concentration of protein in the lens supernatant was determined by the method of Lowry (8). Versitol® (lyophilized human serum) was used as a standard.

A similar technique was employed for determining enzyme activity in erythrocytes. Red cells were washed three times with 0.14 M sodium chloride, and hemolysate was prepared by adding one volume of washed packed red cells to 19 volumes of distilled water. The mixture was centrifuged at 20,000 × g for 20 minutes and the supernatant withdrawn. 0.1 ml. of hemolysate was used for the assay. One unit of enzyme was defined as that amount which reduces 1.0 μmole of triphosphopyridine nucleotide per minute.

RESULTS

Effect of Death on Enzyme Activity of the Lens

Since many of the lens used in this study were obtained at the time of autopsy, the effect of death on enzyme activity in the lens was determined in a group of twelve rabbits. The rabbits were killed by a blow to the occiput, and one eye was removed immediately. The other eye was left *in situ* and the animals were placed in a cold room at 5°C. for varying periods of time: one rabbit for one day, two rabbits for two days, one for three days, two for four days, four for five days, and two for six days. The comparison of glucose-6-phosphate and 6-phosphogluconic dehydrogenase activities between the fresh and post mortem lens is illustrated in Figure 1. No attempt was made to select rabbits of a similar breed, age, or weight, and this may account for the wide range of enzyme levels between different animals. The difference in the enzyme content of the fresh and post mortem lens from the same rabbit, however, never exceeded 20 per cent, and in most animals the values agreed within a range of 10 to 15 per cent. This degree of variation is probably a reflection of the limits of reproducibility of the technique, since as shown in Figure 2, an analogous distribution of results was obtained when assays were done on paired fresh lens. These observations indicate that the activities of lens glucose-6-phosphate and 6-phosphogluconic dehydrogenase are remarkably stable after death.

Studies on Post Mortem Lens

In Table I are represented the results of enzyme assays done on lens obtained at autopsy. Age at the time of death, the weight of the lens, and the soluble protein content of the lens supernate were similar for the four groups of individuals studied. The majority of these patients had not been transfused during the six weeks prior to death, and none of them had received more than two pints of whole blood in this period of time. One Caucasian male and five Negro males demonstrated a marked reduction

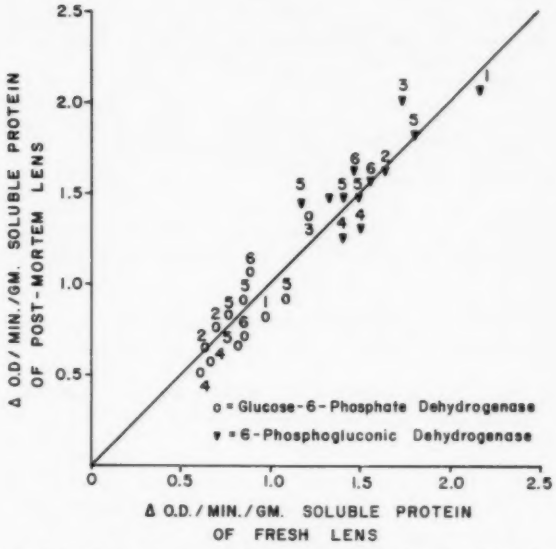
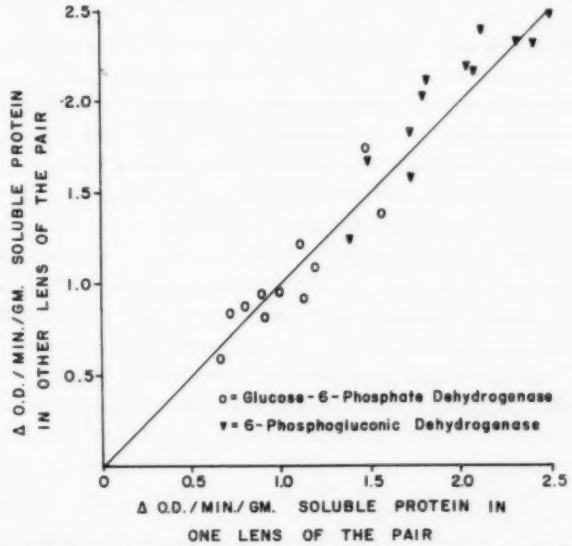

Δ O.D./MIN./GM. SOLUBLE PROTEIN OF POST-MORTEM LENS
Δ O.D./MIN./GM. SOLUBLE PROTEIN OF FRESH LENS
o = Glucose-6-Phosphate Dehydrogenase
▼ = 6-Phosphogluconic Dehydrogenase

FIG. 1. Effect of death on glucose-6-phosphate and 6-phosphogluconic dehydrogenase activities of rabbit lens. The numbers adjacent to each symbol indicate the number of days between death and determination of enzyme activity in the post mortem lens.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Δ O.D./MIN./GM. SOLUBLE PROTEIN IN ONE LENS OF THE PAIR
o = Glucose-6-Phosphate Dehydrogenase
▼ = 6-Phosphogluconic Dehydrogenase

FIG. 2. Comparison of glucose-6-phosphate and 6-phosphogluconic dehydrogenase activities in pairs of fresh rabbit lens.

TABLE I

Glucose-6-Phosphate (G-6-P.D.) and 6-Phosphogluconic (6-PG.D.) Dehydrogenase Activities in Lens and Erythrocytes of Autopsy Cases

Subjects	Number	Age	Weight of Lens (grams)	Protein (mg. per 1.0 ml. homogenate)	Enzyme Studies			
					Erythrocyte		Lens	
					G-6-P.D.*	6-PG.D.*	G-6-P.D.†	6-PG.D.†
White male	31	63	.237 ± .030	30.3 ± 6.9	121 ± 47	122 ± 34	9.4 ± 2.7	2.0 ± 0.6
		(40–94)	(.165–.294)	(21.4–46.0)	(58–267)	(71–260)	(3.8–16.6)	(1.0–4.5)
	1	45	.221	26.1	6	113	1.6	1.4
White female	26	60	.235 ± .032	28.5 ± 5.1	110 ± 29	127 ± 54	9.1 ± 2.6	2.1 ± 0.8
		(39–86)	(.179–.319)	(21.0–39.0)	(59–145)	(86–238)	(4.8–15.9)	(1.2–4.2)
Negro male	30	58	.217 ± .032	29.9 ± 5.4	108 ± 46	128 ± 42	8.7 ± 2.3	2.3 ± 0.7
		(24–85)	(.161–.275)	(20.1–41.0)	(58–293)	(61–228)	(4.4–12.8)	(1.0–4.0)
	1	43	.229	28.6	13	109	2.3	1.6
	1	74	.239	33.0	5	126	2.4	1.8
	1	75	.229	29.0	15	171	1.9	2.1
	1	63	.216	40.5	11	115	2.0	2.2
	1	49	.224	38.0	15	152	2.7	2.6
Negro female	24	59	.226 ± .024	28.6 ± 4.5	108 ± 40	131 ± 53	7.2 ± 2.1	2.0 ± 0.6
		(33–86)	(.168–.267)	(21.0–38.0)	(53–228)	(82–240)	(4.4–11.2)	(1.2–3.1)

* Units of enzyme per 100 ml. red cells. The range of activity for glucose-6-phosphate dehydrogenase in 35 normal living white adult males and females was 130 to 214, and for 6-phosphogluconic dehydrogenase, 129 to 179.

† Δ O.D. per minute per 1.0 gm. of soluble protein.

of lens glucose-6-phosphate dehydrogenase activity, and this was associated with decreased activity of the same enzyme in the red cells. Levels of 6-phosphogluconic dehydrogenase in the lens and erythrocytes of these individuals were comparable to those observed in subjects with normal red cell glucose-6-phosphate dehydrogenase activity.

An attempt was made to study the relatives of those individuals in whom the activities of red cell and lens glucose-6-phosphate dehydrogenase were reduced. To date the blood from only two key relatives has become available for study: the mothers of two Negro males. Both of these women had a partial deficiency of glucose-6-phosphate dehydrogenase in their erythrocytes. The mother of the Caucasian male with diminished red cell and lens glucose-6-phosphate dehydrogenase activity was Italian, a racial group in which the primaquine-sensitive defect has been defined.

Studies on Cataractous Lens

The results of enzyme studies on cataractous lens are listed in Table II. All of these patients had senile cataracts with varying degrees of opacifica-

TABLE II

Glucose-6-Phosphate and 6-Phosphogluconic Dehydrogenase Activities in Lens and Erythrocytes from Patients with Cataracts

Subjects	Number	Age	Weight of Lens (grams)	Protein (mg. per 1.0 ml. homogenate)	Enzyme Studies			
					Erythrocyte		Lens	
					G-6-P.D.*	6-PG.D.*	G-6-P.D.*	6-PG.D.*
White male	20	67 (53–85)	.192 ± .028 (.136–.272)	24.5 ± 6.2 (13.8–35.2)	189 ± 25 (141–243)		9.2 ± 3.1 (4.7–14.3)	1.9 ± 0.4 (1.1–2.5)
White female	21	65 (53–85)	.195 ± .037 (.142–.299)	25.4 ± 5.9 (11.8–35.4)	184 ± 20 (149–221)		8.2 ± 3.5 (5.0–20.8)	1.8 ± 0.5 (1.1–3.7)
Negro male	16	63 (47–80)	.181 ± .024 (.157–.213)	17.4 ± 7.6 (4.2–33.5)	191 ± 21 (136–246)		15.7 ± 5.4 (6.5–32.5)	4.8 ± 0.9 (1.6–11.3)
	1	80	.176	10.4	18	163	4.2	3.3
	1	47	.182	30.8	3	162	2.8	2.0
Negro female	12	65 (39–82)	.196 ± .026 (.133–.280)	24.2 ± 8.4 (2.7–38.5)	168 ± 27 (128–209)		13.1 ± 6.0 (5.8–50.6)	3.3 ± 0.8 (2.0–10.7)

* Units of enzyme activity are similar to those presented in Table I.

tion of the cortex, the nucleus, or the entire lens. Although the distribution of subjects by age was approximately the same as that observed in the autopsy group, the weight and soluble protein content of the lens was reduced. The magnitude of the decrease was related to the clinical maturity of the cataract; as the intensity of the sclerotic change increased, the weight and soluble protein content of the lens decreased.

Specific activities of glucose-6-phosphate and 6-phosphogluconic dehydrogenase were also affected by the maturity of the cataract, the highest activity being observed in the furthest advanced cataracts.[2] In one Negro female, for example, the concentration of soluble protein in the lens supernate was 2.7 mg. per cent, while the specific activities of glucose-6-phosphate and 6-phosphogluconic dehydrogenase were 50.6 and 10.7 respectively. The enzyme content of lens from patients with incipient or immature cataracts was either the same, or in some instances slightly higher than the values obtained in autopsy lens. Since the majority of the Negro patients had mature cataracts, and most of the Caucasians presented with immature cataracts, enzyme levels were significantly greater in the Negroes.

As shown in Table II, cataractous lens from two Negro males with primaquine-sensitive erythrocytes were studied. One, the eighty-year-old man,

[2] A similar correlation was demonstrated when the wet weight of the lens in grams was used as a standard of reference for enzyme activity.

had a mature cataract; the cataractous change in the other was of the immature variety. The activity of glucose-6-phosphate dehydrogenase was definitely decreased in both lens, whereas the level of 6-phosphogluconic dehydrogenase was normal. Reduction of glucose-6-phosphate dehydrogenase activity was less marked in the lens from the eighty-year-old man with the mature cataract. This indicates that the enzyme content of cataractous lens from primaquine-sensitive individuals may increase as the cataract matures. The extent of the increase, however, does not parallel that observed in cataractous lens from persons with normal glucose-6-phosphate dehydrogenase activity of the red cell.

DISCUSSION

Marks, Gross, and Hurwitz were the first to report on the anatomical distribution of the enzyme abnormality in Negroes with primaquine-sensitive erythrocytes (3). After assaying for enzyme activity in the leukocytes and livers of affected subjects, they concluded that the defect was restricted to the erythrocytes. More recent observations by Okita and Carson (1), however, indicated that the enzymatic abnormality involved other tissues. In primaquine-sensitive Negroes they found that approximately 5 per cent of parenterally administered glucose-1-C^{14} was converted to $C^{14}O_2$ during a period of two hours following the injection. In non-sensitive subjects 10 per cent of the glucose-1-C^{14} was transformed to $C^{14}O_2$.

In the present study a significant lowering of glucose-6-phosphate dehydrogenase activity was demonstrated in cataractous and post mortem lens of Negroes with primaquine-sensitive erythrocytes. Since it was impossible to obtain fresh normal human lens, an important consideration is whether or not decreased glucose-6-phosphate dehydrogenase activity was a consequence of death or the cataractous process. In rabbits glucose-6-phosphate and 6-phosphogluconic dehydrogenase activities of the lens were stable for as long as six days after death. In normal humans, progression of the cataract, as measured by decreased concentration of soluble protein, was accompanied by an increase in the specific activity of the two enzymes. Hence a lowering of glucose-6-phosphate dehydrogenase activity could not be attributed to post mortem changes or the metabolic alterations associated with cataract formation. Furthermore 6-phosphogluconic dehydrogenase activity in the lens of the primaquine-sensitive subjects was normal, or even slightly increased, indicating that the abnormality of glucose-6-phosphate dehydrogenase was not a manifestation of a generalized depression of enzyme activity.

That the reduction of lens glucose-6-phosphate dehydrogenase activity accompanied a genetically determined deficiency of the same enzyme in

the erythrocytes is based on the following observations. Enzyme assays on red cells from the cataractous patients were performed on freshly drawn blood, and although some of these patients had associated medical illnesses, none had diseases which are known to affect glucose-6-phosphate dehydrogenase activity of the erythrocyte. It is concluded, therefore, that the marked reduction of glucose-6-phosphate dehydrogenase levels in red cells and lens of the two Negro males with cataracts can be attributed only to the primaquine-sensitive type of abnormality.

Identification of the primaquine-sensitive subjects in the post mortem group was complicated by the broad range of enzyme values observed in red cells obtained at autopsy. This marked variability could have been related to one of several factors; e.g., administration of foreign blood prior to death, or the effect of post mortem changes on red cell enzyme activity. The first of these possibilities was controlled by using patients who had received no more than two pints of blood during the four-week period prior to death; and in fact, the majority of these patients were given no transfusions. As for the second possibility, death was associated with a variety of conditions; carcinomas of the breast, bladder, cervix, lungs, or gastrointestinal tract, brain tumors, cerebrovascular accidents, myocardial infarctions, collagen vascular diseases, malignant hypertension, renal failure, and a variety of infections. None of these disorders was accompanied by a characteristic change in the enzyme pattern of the red cells. Patients with cerebrovascular accidents, for example, had depressed, normal, or elevated enzyme activity. Also in most of the patients the increments or decrements of glucose-6-phosphate dehydrogenase activity paralleled those for 6-phosphogluconic dehydrogenase. The only exceptions were the one Caucasian and five Negro males who were primaquine-sensitive. In these individuals a marked reduction of red cell glucose-6-phosphate dehydrogenase was accompanied by normal 6-phosphogluconic dehydrogenase activity.

A positive identification of the post mortem primaquine-sensitive subject could also have been established through complete family studies. None of the daughters and only two of the mothers of the affected males were available. Both of these women demonstrated a partial deficiency of red cell glucose-6-phosphate dehydrogenase and were considered to be heterozygous for the primaquine-sensitive defect. Although this evidence does not prove the presence of the abnormality in their sons, at least it indicates an appropriate genetic background for its occurrence.

Very little is known about the synthesis and degradation of glucose-6-phosphate dehydrogenase by the human lens. The enzyme protein is undoubtedly synthesized in the single layer of epithelial cells beneath the anterior capsule. Since most of the glucose-6-phosphate dehydrogenase ac-

tivity is present in the cortical portion of the lens, a constant replenishment or activation of the enzyme in these areas is essential for normal enzymic activity. Which one of these factors is defective in primaquine-sensitive lens is not known.

Another unanswered question is why the enzymatic defect involves the red cell and the lens, but not the leukocytes or the liver. A possible explanation for this type of distribution is based on the assumption that glucose-6-phosphate dehydrogenase exists in multiple molecular forms. There are many examples of the molecular heterogeneity of enzymes in one organism, and even in one tissue (9, 10). Although evidence for the heterogeneity of glucose-6-phosphate dehydrogenase in rat tissues has been presented by Moore (11) studies of a similar nature are not available in humans. If glucose-6-phosphate dehydrogenase does exist in several molecular forms, then a genetic defect of the enzyme may affect only one of these species. Consequently the enzymatic abnormality would be evident only in tissues containing the isozyme whose activity is controlled by the mutant gene. It is proposed, therefore, that the lens and erythrocyte may have a similar complement of isozymes for glucose-6-phosphate dehydrogenase, and that the isozyme affected in primaquine-sensitive individuals accounts for most of the enzyme activity in these tissues.

Even though the factors effecting a reduction of glucose-6-phosphate dehydrogenase in the lens of primaquine-sensitive persons are unknown, it is evident that the enzyme abnormality of the lens is not as marked as that observed in the red cell. Furthermore, heterozygous Negro females with a partial deficiency of lens glucose-6-phosphate dehydrogenase were not detected in this study. These results may be secondary to the capacity of the mature lens to synthesize enzyme protein.

Opacification of lens fibers can follow exposure to a wide variety of physical and chemical agents, e.g., naphthalene, alloxan, x-ray, and dinitrophenol, or it may be an accompaniment of senescence. At the present time it is unclear whether the deficiency of lens glucose-6-phosphate dehydrogenase predisposes primaquine-sensitive persons to the formation of either type of cataract. Several observations suggest that it might. Many investigators have noted a decrease in the concentration of reduced glutathione preceding the formation of lens opacities (12, 13). Also it has been proposed that reduced glutathione is an important regulatory factor in controlling the rate of oxidation, as well as the degree of solubility of lens proteins (14). Recently, depressed glucose-6-phosphate dehydrogenase activity has been observed in the early phases of galactose-induced cataracts in rats (15). Although these metabolic aberrations may not be directly related to the production of opaque lens fibers, their occurrence indicates a possible relationship between activity of the hexose-monophosphate

shunt and the pathogenesis of cataracts. Furthermore, they suggest that a person with a genetically determined deficiency of glucose-6-phosphate dehydrogenase may be more susceptible to cataract formation. The number of observations in the present study are too few to determine if senile cataracts occur more frequently in primaquine-sensitive individuals. Even less information is available on the incidence of chemically induced cataracts. A definitive demonstration of a causal relationship between the metabolic defect of primaquine-sensitivity and the formation of cataracts will have to await the results of future studies.

SUMMARY

Activities of glucose-6-phosphate and 6-phosphogluconic dehydrogenase were determined in erythrocytes and lens from American Negroes and Caucasians with senile cataracts. Two primaquine-sensitive Negro males manifested a significant reduction of glucose-6-phosphate dehydrogenase activity in their lens. The level of 6-phosphogluconic dehydrogenase was normal.

Similar studies were carried out on lens and red blood cells from another group of Negroes and Caucasians at the time of autopsy. One Caucasian male and five Negro males with marked reduction of red cell glucose-6-phosphate dehydrogenase activity demonstrated decreased activity of the enzyme in the lens. A genetic background for the primaquine-sensitive type of abnormality was delineated in two of the Negro males.

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THE HOMOZYGOUS STATE OF PERSISTENT FETAL HEMOGLOBIN AND THE INTERACTION OF PERSISTENT FETAL HEMOGLOBIN WITH THALASSEMIA[1]

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INTRODUCTION

Edington and Lehmann (1) described a condition in which high proportions of fetal hemoglobin were encountered in the blood of healthy negro adults. Jacob and Raper (2) suggested the name "hereditary persistence of fetal hemoglobin." This condition has been shown to be allelic with hemoglobins S (3, 4, 5) and C (6). The abnormality appears to affect the synthesis of beta chains of the hemoglobin molecule. Interaction between hereditary persistence of fetal hemoglobin and beta chain thalassemia has been noted (6, 7). Previous reports from this laboratory (3, 4, 5) have dealt with the nature of the alkali resistant hemoglobin, the frequency of the anomaly, the mode of inheritance, and the interaction between persistent fetal hemoglobin and sickle cell trait. This paper presents a study of members of a family in whom both persistent fetal hemoglobin and thalassemia have been found. One individual appears to be homozygous for hemoglobin F. In addition, further observations have been made on the inheritance and clinical implications of persistent fetal hemoglobin.

METHODS

Hematocrit and hemoglobin determinations, white blood cell, red blood cell, reticulocyte, and platelet counts were performed in the research laboratories of the hematology division by carefully standardized methods. Red blood cell counts were performed in the Coulter electronic red blood cell counter. Hemoglobin determinations were done in duplicate using a Beckman Model B spectrophotometer and calibrated with a cyanmethemoglobin standard (8). Osmotic fragility of the red cells was studied by the method of Emerson (9). Hemoglobin electrophoreses were performed on paper (10), agar gel (11), and starch block (12). The concentration of hemoglobin F (alkali resistant hemoglobin) was deter-

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mined by a modification (13) of Singer's method (14). The hemoglobin binding capacity of serum, the serum haptoglobin, was estimated using a technique described by Herman (15).

Study of a Family in which Hereditary Persistence of Fetal Hemoglobin and Thalassemia Coexist

Propositus

R.M. (JHH #842064), a twenty-year-old negro woman, was seen in the obstetric clinic of The Johns Hopkins Hospital for a routine prenatal visit during her second pregnancy. As part of a screening study for abnormal hemoglobins, a specimen of blood was sent to the hematology laboratory for paper electrophoresis. There was no family history of anemia, and all her siblings were living and well. The patient had had a completely uneventful past history. She had been treated with iron during her first pregnancy at which time her hematocrit value had been recorded as 34, 33, and 31 per cent, values which are not unusual in pregnant women in this clinic. She delivered the first child in September, 1959. The expected date of confinement for the second pregnancy was December, 1960. Physical examination in July, 1960, revealed a well-developed female with normal vital signs and an enlarged uterus commensurate with the duration of gestation. The mucous membranes were of good color, and there was no enlargement of the liver or spleen. Because of the demonstration of a slow-moving hemoglobin electrophoretic component on paper similar to that seen in umbilical cord blood of newborn infants, other studies were done and are shown in Table I (II-11). The hematocrit value was 39 per cent. There was an increase in the number of reticulocytes, a slight bilirubinemia, absent haptoglobin, and increased resistance to osmotic lysis of the red blood cells. Erythrocyte morphology was greatly altered with marked microcytosis, anisocytosis, and polikilocytosis. Many target cells were present (Fig. 4a). Fetal hemoglobin was found to be 69 per cent, and hemoglobin A_2 was 2.3 per cent.

Family Study

The family pedigree is shown in Figure 1. Hematologic values are summarized in Table I. The patient's father (I-2) was found to have 25 per cent fetal hemoglobin and a normal value for A_2 hemoglobin. Hematocrit, MCV, MCHC, haptoglobin, and osmotic fragility were normal. Red blood cell morphology was not remarkable. This is the pattern of hereditary persistence of fetal hemoglobin (A-F). The mother (I-3) of the propositus was found to have a normal amount of fetal hemoglobin, but the value of A_2 hemoglobin was 5.3 per cent. The MCV of her red blood cells was 81.5 μ^3, and the blood smear was normal except for mild microcytosis. The osmotic fragility of the red blood cells after incubation showed increased resistance to lysis. Other hematologic values were normal. This is consistent with the diagnosis of thalassemia trait (A-Thal).

The siblings of the propositus were studied, and it was found that three brothers (II-5, II-6, and II-7) also had values for hemoglobin F in the

range of 67 to 70 per cent, but none was anemic. One brother (II-5) had evidence of a mild compensated hemolytic process manifested by increase in the bilirubin and lowered haptoglobin. All three had microcytosis, slight elevation of the reticulocytes, increased resistance of the erythrocytes to osmotic lysis, and morphologic abnormalities of the red blood cells similar to those of the propositus. The values for hemoglobin A_2 ranged from 2.5 to 3.0 per cent. These three individuals and the propositus appear to be heterozygous for both hereditary persistence of fetal hemoglobin and thalassemia (F-Thal).

Two other siblings (II-8 and II-9) were found to have normal values for hemoglobin F but elevated concentrations of A_2 hemoglobin (5.7 and 5.1 per cent). Although neither was anemic, both showed microcytosis. One of the half-siblings (II-3) of the propositus showed similar changes with A_2 hemoglobin of 5.0 per cent. She also demonstrated a slight elevation of the fetal component (3.2 per cent). These individuals have thalassemia trait.

A sister (II-10) and a half-brother (II-2) showed normal values for both fetal hemoglobin and hemoglobin A_2 and no abnormalities in erythrocyte morphology. A half-sister (II-1) had a normal value for hemoglobin F, the A_2 fraction not being determined. She had severe menorrhagia, and her red blood cells were hypochromic and microcytic.

All of the children of one brother (II-5) with 70 per cent fetal hemoglobin were studied. Three (III-1, III-3, and III-4) were found to have elevated values for hemoglobin A_2 and normal concentrations of fetal hemoglobin while one had normal A_2 and 28 per cent fetal hemoglobin (III-2).

The husband of the propositus (II-12), his father (I-5), and two sisters (II-16 and II-17) were found to have fetal hemoglobin values between 21 and 27 per cent. None was anemic or symptomatic, and blood smears of these individuals were normal. Hemoglobin A_2 was determined in the patient's husband and was 1.1 per cent.

The one year old child (W.M. III-5) of the propositus demonstrated alkali resistant hemoglobin in the range of 93 to 96 per cent, but only hemoglobin F could be demonstrated on paper, agar gel, starch block, and starch gel electrophoresis and on column chromatography.[3]

The agar gel patterns shown in Figure 2 demonstrate the absence of hemoglobin A. Electrophoresis on starch block (Fig. 3) demonstrates the

[3] Column chromatography and starch gel electrophoresis were kindly performed by Dr. Corrado Baglioni of the Massachusetts Institute of Technology. Starch gel electrophoresis was also performed by Dr. David Weatherall of The Johns Hopkins University. Paper, agar gel, and starch block electrophoresis were performed in this laboratory.

TABLE I

Hematologic Values in the Family of the Propositus R.M. II-11

Pedigree Designation	Initial, Age, Sex	Fetal Hemoglobin (%)	As Hemoglobin (%)	Hematocrit Value (%)	Red Cell Count (Millions/mm.³)	Hemoglobin (gm. %)	Reticulocytes (%)	M.C.V. (μ3)	M.C.H.C. (%)	Haptoglobin	Bilirubin (mg. %)	Osmotic Fragility		Erythrocytic Morphology							
												Non-Incubated	Incubated	Anisocytosis	Poikilocytosis	Microcytosis	Polychromatophilia	Target Cells	Spherocytes	Nucleated R.B.C.	Achromia
I-2	T.B. 60 M	25.0	1.5	45	4.7	14.6	1.2	96	32	normal	—	normal	normal	0	0	0	0	0	0	0	0
I-3	L.M.B. 51 F	1.1	5.3	41	5.1	13.3	1.4	80	32	normal	—	normal	slightly increased resistance	0	0	+	0	0	0	0	0
I-5	W.M. 53 M	24.6	—	53	—	—	—	—	—	—	—	—	—	0	0	0	0	+	0	0	0
II-1	M.S. 35 F	<3*	—	33	—	9.8	—	—	30	—	—	—	—	++	++	+	0	++	0	0	+
II-2	G.B. 31 M	0.7	2.3	48	—	16.6	—	—	35	—	—	—	—	0	0	0	0	0	0	0	0
II-3	B.K. 34 F	3.2	5.0	36	—	11.5	—	—	32	—	—	—	—	+	0	+	0	+	0	0	±
II-5	Je.B. 28 M	70.7	2.5	47	5.9	15.3	3.8	80	33	absent†	2.8	slightly increased resistance	increased resistance	+++	++	+	++	+++	++	0	0
II-6	A.B. 29 M	66.8	2.7	41	5.3	13.3	3.2	77	32	normal	1.1	slightly increased resistance	increased resistance	++++	+++	++	++	+++	++	0	0
II-7	T.B. 16 M	68.4	3.0	41	5.3	13.2	3.3	77	32	normal	0.9	slightly increased resistance	increased resistance	+++	++	++	++	+++	++	0	0
II-8	Jo.B. 24 M	1.6	5.7	45	6.1	13.8	2.5	74	31	—	<0.8	—	—	+	+	++	+	0	0	0	0
II-9	L.B.H. 20 F	1.0	5.1	40	—	12.9	—	—	32	—	—	—	—	+	0	+	0	+	0	0	0
II-10	R.H. 26 F	0.8	2.5	43	—	14.4	—	—	34	—	—	—	—	0	0	0	0	0	0	0	0
II-11	Re.M. 20 F	69.0	2.3	39	5.8	13.0	4.8	67	33	absent†	2.0	slightly increased resistance	increased resistance	++++	+++	+++	++	+++	++	0	0
II-12	Ru.M.	26.0	1.1	46	5.2	15.0	1.1	89	33	—	—	—	—	+	0	0	0	+	0	0	0

	20 F											creased resistance	increased resistance	++++	+++	+++	++	+++	++	0	0
II-12	Ru.M. 26 M	26.0	1.1	46	5.2	15.0	1.1	89	33	—	—	—	—	+	0	0	0	+	0	0	0
II-13	W.M. 23 M	<3*	—	46	—	—	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0
II-14	E.M. 24 M	<3*	—	53	—	—	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0
II-15	M.W. 28 F	<3*	—	42	—	—	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0
II-16	M.M. 30 F	21.4	—	41	—	—	—	—	—	—	—	—	—	+	+	0	0	0	0	0	0
II-17	L.M.M. 20 F	26.2	—	—	—	—	—	—	—	—	—	—	—	0	0	0	+	0	0	0	0
III-1	J.B. 8 M	1.8	5.1	37	4.5	11.8	1.7	82	32	—	—	—	—	++	+++	+	+	±	0	0	±
III-2	R.B. 7 F	28.2	1.6	42	5.2	14.1	1.3	81	34	—	—	—	—	0	0	±	0	0	0	0	0
III-3	V.B. 5 F	2.1	4.8	34	4.4	10.9	1.2	77	32	—	—	—	—	+	++	+	0	0	0	0	0
III-4	E.B. 3 F	2.2	5.3	40	5.3	11.5	1.0	76	29	—	—	—	—	+	+	++	0	0	0	0	+
III-5	W.M. 15 mo. M	>90	<0.05	25	4.1	5.3	2.5	61	21	normal	<0.8	increased resistance	increased resistance	++++	++++	++++	++	++++	++	0	++++
III-5	W.M. 20 mo. M p 30 days Fe Rx.	>90	<0.05	43	6.1	13.3	2.7	71	31	—	—	—	—	+++	+++	++	+	++++	++	0	±
III-6	R.M. birth M	75.0	0.3	50	4.3	16.7	6.3	116	33	—	—	—	—	—	—	—	—	—	—	—	—
III-6	R.M. 5 mo. M	10.4	4.3	42	5.6	12.3	0.9	75	29	—	—	—	—	+++	+++	++	0	+	0	0	+

*—within normal limits by unmodified technique of Singer (14).
†—less than 5 mg.% expressed as hemoglobin binding capacity.

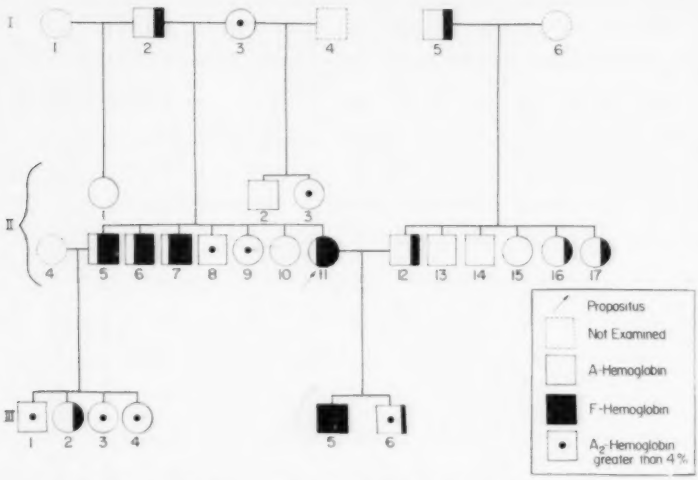

FIG. 1. Hemoglobin phenotypes of the family of the propositus R.M. (II-11).

absence of hemoglobin A_2. On the basis of these data it is presumed that this individual is homozygous for hereditary persistence of fetal hemoglobin (F-F).

W.M. (III-5) was 15 months old when he was first studied. He was the product of a normal labor and delivery. There was no history of illness except for an occasional common cold. His growth and development had been normal. There was no history of dietary inadequacy other than an aversion to meat. Physical examination revealed pallor of the mucous membranes in an otherwise robust and well-developed child without hepatomegaly or splenomegaly. The child was found to be anemic with an hematocrit value of 25 per cent. There was marked anisocytosis, poikilocytosis, leptocytosis, hypochromia (MCHC 21 per cent), and microcytosis. There were many polychromatophilic red blood cells, moderate numbers of spherocytes, but no normoblasts were seen. Osmotic fragility studies revealed increased resistance to lysis (0.35 to 0.15) before incubation and increased resistance (0.45 to 0.20) after incubation at 37 degrees centigrade for 24 hours. There was a mild increase in the number of reticulocytes, and the serum bilirubin and serum haptoglobin were normal. It was felt that some of these changes might be the result of iron deficiency, and he was treated with ferrous sulfate, 600 milligrams per day, for thirty days. At the end of this time his hematocrit had risen to 45 per cent with improvement of the MCV and MCHC values. The amount of alkali-resistant hemoglobin remained unchanged. His blood was studied again

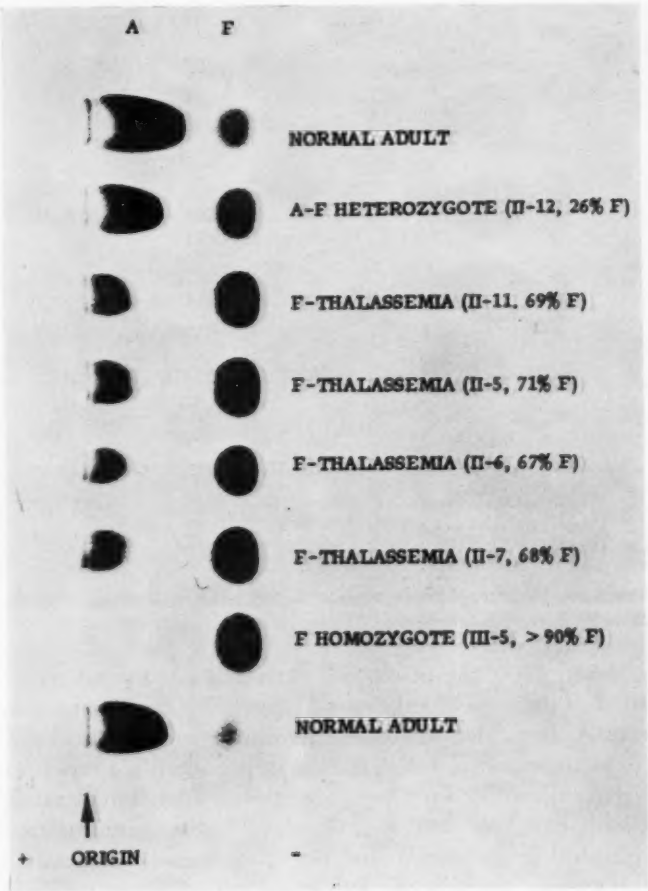

FIG. 2. Electrophoretic patterns on agar gel (citrate buffer, pH 6.0) of hemoglobins of family of the patient, R.M. Note the absence of hemoglobin A in the F homozygote. Lower concentrations of hemoglobin A migrate slower in this medium. The lowest pattern (normal adult) demonstrates the appearance of a dilute sample on agar gel.

at 120 days after the conclusion of the iron therapy at the age of twenty months. *No hemoglobin component other than fetal hemoglobin could be demonstrated.* The hematocrit was 43 per cent, and the red blood cell indices indicated microcytosis without hypochromia. The erythrocyte morphology was still abnormal with numerous target cells, moderate to marked anisocytosis and poikilocytosis, and a moderate number of spherocytes (Fig. 4b). The child has remained clinically well.

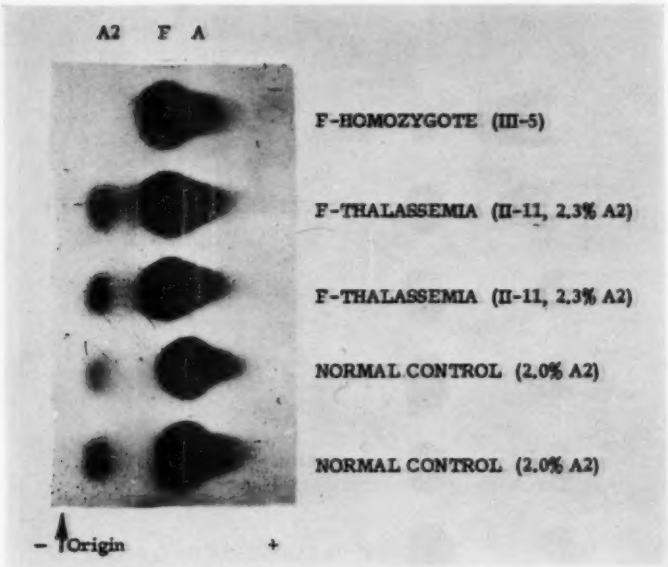

FIG. 3. Starch block electrophoresis (barbital buffer, pH 8.6) demonstrating the absence of hemoglobin A_2 in the F homozygote.

In December, 1960, the propositus delivered her second child (III-6). Examination of the cord blood showed 75 per cent fetal hemoglobin with 0.3 per cent A_2 hemoglobin. At two months of age the child showed 63 per cent fetal hemoglobin, and at the age of five months a repeat examination showed a value of 10.4 per cent hemoglobin F with an elevation of the A_2 hemoglobin to 4.3 per cent. Red blood cell indices demonstrated microcytosis with mild hypochromia, and the smear showed moderate anisocytosis, poikilocytosis, microcytosis, and an occasional target cell. This child appears to have thalassemia trait (A-Thal).

The levels of hemoglobin A_2 in members of this family with A-thalassemia and F-thalassemia are compared to those of a group of normal persons (A-A) and individuals with hereditary persistence of fetal hemoglobin (A-F) (Fig. 5). A pronounced increase in hemoglobin A_2 was found only in the A-thalassemia group.

Survey of a Negro Population

The survey to determine the frequency of elevations of fetal hemoglobin initiated by Bradley and his associates (5) has been continued. An additional 3000 adult negro patients have been screened. Three individuals with hereditary persistence of fetal hemoglobin were discovered. In

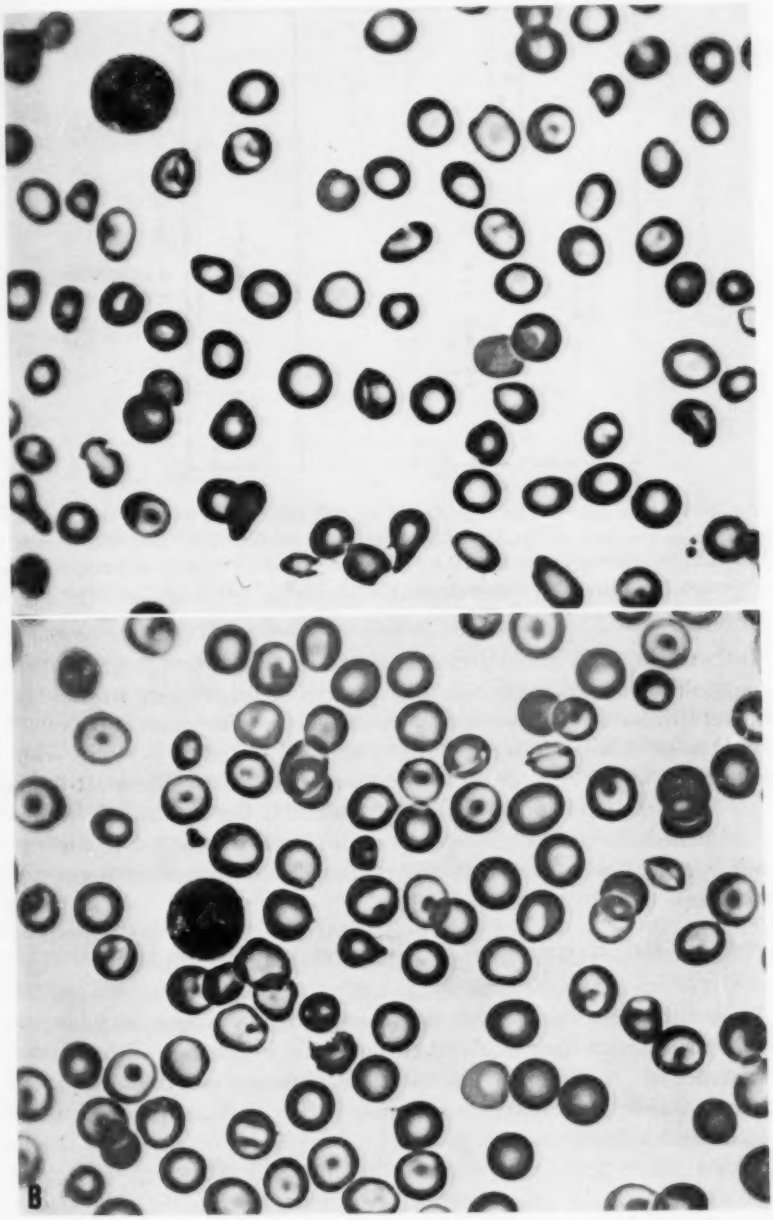

FIG. 4. Photomicrograph (×1000) of the red blood cells (a) of the propositus, F-thalassemia (R.M. II-11) and of her son (b) the F homozygote (W.M. III-5).

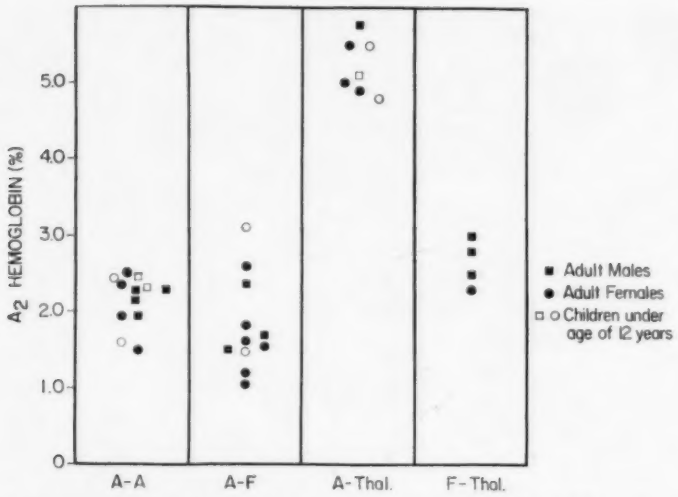

FIG. 5. Percentage of A_2 hemoglobin in normal individuals (A-A), heterozygotes for hereditary persistence of fetal hemoglobin (A-F), persons with thalassemia trait (A-Thal), and the heterozygotes with both hereditary persistence of fetal hemoglobin and thalassemia (F-Thal). This demonstrates that A_2 hemoglobin is elevated only in those persons with thalassemia trait.

a study of the families of these persons, nineteen other people with A-F hemoglobin were encountered. The pattern of inheritance in these families was similar to that previously described (5). The percentage of F hemoglobin in these individuals ranged from 21.4 to 29.3 per cent with an average of 26.3 per cent. None of these persons has had illness attributable to the abnormal hemoglobin. Figure 6 depicts the hematocrit values of these individuals compared to persons with hemoglobin A-A in these same families and shows that there was no significant difference between the two groups.

Elevated Fetal Hemoglobin in Disorders other than Hereditary Persistence of Fetal Hemoglobin

In our total survey of 5000 negro patients, elevations of fetal hemoglobin to levels as high as those encountered in persons with hereditary persistence of fetal hemoglobin were found in six individuals. Values of this magnitude were encountered only in thalassemia major, sickle cell disease, and aplastic anemia.

DISCUSSION

The hereditary persistence of fetal hemoglobin is a benign abnormality in which a peculiar hemoglobin apparently identical to the fetal hemo-

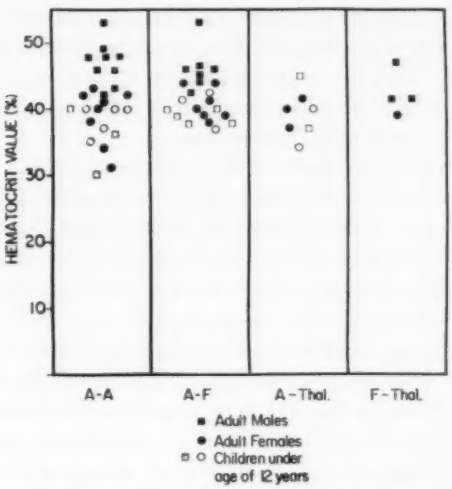

FIG. 6. Hematocrit values of persons with A-A, A-F, A-Thal, and F-Thal demonstrating no significant difference between the groups.

globin of the newborn child persists in high concentrations in the adult. The family study described demonstrates the interaction of hemoglobin F with thalassemia, and one individual (W.M. III-5) appears to be homozygous for hemoglobin F.

Individuals with F-thalassemia do not have increased morbidity when compared to individuals in the same family who have A-thalassemia. The interaction of these two genetic anomalies does produce more striking red blood cell abnormalities, and in two persons there is evidence of a compensated hemolytic process. The child with homozygous F hemoglobin was anemic when first studied, presumably on the basis of dietary iron lack. When he was given iron his hematocrit returned to normal in the expected period of time, indicating that he was able to synthesize hemoglobin rapidly. This patient has striking red blood cell abnormalities consisting of microcytosis, anisocytosis, poikilocytosis, target cells, and occasional spherocytes (Fig. 4b), but he has no anemia or apparent clinical disease.

Individuals who are heterozygous for F hemoglobin (A-F) have no demonstrable illness. Red cell abnormalities in these persons have been variable, and when present, consist of mild to moderate increase in the number of target cells. This mild abnormality has been noted in five of twenty-two individuals with A-F. In a previous study reported by Bradley and Conley (4), no consistent deviation from normal in the microscopic appearance of the red cells was noted in twenty-eight individuals with

A-F. Kraus *et al.* (6) noted increased target cells in three of eight individuals with A-F. Bradley and Conley noted no anemia in their group, and as depicted in Figure 6, there was no suggestion of anemia in persons of the present study with A-F hemoglobin when compared with results obtained from family members with normal hemoglobin.

In a survey of two thousand adult negroes in Baltimore (5), two persons were encountered with a striking increase of hemoglobin F in the order of 25 per cent. Observations on an additional 3000 negroes have been made, and three individuals with hereditary persistence of fetal hemoglobin have been found. The incidence of this condition appears to be of the order of 1 in 1000 Baltimore negroes.

In this family (Fig. 1) thalassemia trait is manifested by microcytosis and an elevation of the hemoglobin A_2 component. This variety of thalassemia has been shown to be allelic with other abnormalities of the beta chain of the hemoglobin molecule (16, 17). Hereditary persistence of fetal hemoglobin also behaves as a mutation of the beta chain gene. It has been shown to be allelic with hemoglobins S (3, 4, 5) and C (6).

The heterozygote A-F and the heterozygote of this type of thalassemia (A-Thal) have certain common findings: both are genetic defects which affect the synthesis of the beta chain of the hemoglobin molecule, the two are allelic, and there is a variable change in the erythrocyte morphology. On the other hand the homozygotes of the two conditions differ markedly. There is no splenomegaly, anemia, or normoblastosis in the homozygote of persistent fetal hemoglobin (F-F), and there appears to be a total suppression of beta chain and delta chain synthesis in the F-F individual with replacement by gamma chains in the hemoglobin to bring forth 100 per cent F hemoglobin, since neither A hemoglobin nor A_2 hemoglobin could be demonstrated. This suppression is partial in thalassemia major and variable amounts of A hemoglobin and A_2 hemoglobin are produced. Furthermore, in hereditary persistence of fetal hemoglobin there is no elevation of the A_2 component in the heterozygote nor when combined as F-thalassemia (Fig. 5). In thalassemia major, hemoglobin synthesis is depressed but the homozygous F individual can synthesize hemoglobin rapidly and in normal amounts as shown by the response to iron therapy in the patient W.M. III-5.

Increased amounts of fetal hemoglobin can be produced in a variety of circumstances both environmental and under genetic control. Although the fetal hemoglobins produced under these conditions appear to be identical, a recent report has questioned their homogeneity (18). However, the fetal hemoglobin of hereditary persistence of fetal hemoglobin has been compared by a variety of techniques with the fetal hemoglobin of cord blood and no differences were demonstrated (2, 5). In some instances

it would appear as if the production of fetal hemoglobin is a compensatory mechanism which brings forth hemoglobin F when the synthesis of hemoglobin A is deficient or absent (19). Thus in the heterogenous group of conditions such as aplastic anemia, homozygous sickle cell anemia, and thalassemia major, fetal hemoglobin concentrations vary greatly within each disorder. In hereditary persistence of fetal hemoglobin, however, the heterozygotes (A-F) have fetal hemoglobin levels which fall within a fairly narrow and constant range (18 to 33 per cent) with a mean of 26.3 per cent in the fifty studied at this institution.

In the homozygous state (F-F) there does not appear to be impressive alteration of total hemoglobin synthesis. However, when the synthesis of F hemoglobin is compared to the synthesis of other hemoglobins in heterozygous states, an apparent order of efficiency of production is established. In the combination A-F, approximately 75 per cent of the hemoglobin is hemoglobin A. In the combinations A-S and A-C hemoglobin, A accounts for only 60 per cent of the total hemoglobin. Similarly, when F is combined with S hemoglobin (S-F) 70 per cent of the hemoglobin is hemoglobin S, and in C-F the percentage of C is approximately 63 per cent. Thus the genetic mechanism which produces the F hemoglobin does not appear to be as efficient as that which produces hemoglobins A, S, and C.

In the heterozygote F-Thalassemia, hemoglobin F is present in levels of approximately 68 per cent. Similar percentages of hemoglobins S and C may be found when each of these is in combination with thalassemia trait (S-Thal and C-Thal). A comparable situation with respect to alpha chain abnormalities has been described in the heterozygote, hemoglobin I-alpha thalassemia, in which 70 per cent of the hemoglobin is hemoglobin I (20).

A number of genetic mechanisms for the production of hemoglobin F have been suggested. Zuelzer *et al.* (21, 22) have drawn attention to the reciprocal relationship of hemoglobin A_2 and F in beta chain thalassemia and have postulated that in this situation the gamma chains of fetal hemoglobin are in essence primitive beta chains which have been "blocked." They did not apply this mechanism to the inheritance of persistent fetal hemoglobin but felt that the high percentage of hemoglobin F in this syndrome represented a back mutation of a beta chain gene. It has been suggested that a "switching" mechanism exists which controls the production of hemoglobin A after birth (22). The fact that hemoglobin A production begins early in fetal life (23), that hemoglobin F is produced in gradually decreasing amounts until after the first year of life, and that the ability to make fetal hemoglobin is retained in the adult would point to a "rheostat" mechanism rather than a "switch."

The data from the families in this report strongly suggest that the production of F hemoglobin in the inherited persistence of fetal hemoglobin

is a result of a mutation at the locus which controls beta chain synthesis. In contrast with thalassemia, there is no suggestion of deficient amount of hemoglobin synthesis in the heterozygote (A-F) or the homozygote (F-F). In addition, individuals with S-F and C-F do not have evidence of the clinical disorders encountered in individuals with S-thalassemia and C-thalassemia. The effect of the mutation which produces persistent fetal hemoglobin either specifically directs the formation of hemoglobin F or prevents any formation of normal beta or delta chains allowing gamma chain production to continue.

This last mechanism is an attractive hypothesis. Normally the genes for A hemoglobin are present in the homozygous state. When one gene for abnormal hemoglobin production is present, more than 50 per cent of the hemoglobin is hemoglobin A suggesting that a single gene for hemoglobin A production may direct the synthesis of approximately 70 per cent of the total hemoglobin production. The balance is made up by the abnormal hemoglobin. This situation is seen in sickle trait and C-trait (A-S and A-C). In A-F the one A gene directs the synthesis of 75 per cent of the total hemoglobin production, and the remainder is made up of F hemoglobin because the mutation for persistent fetal hemoglobin prevents the formation of beta chains and delta chains, and the amount of F hemoglobin represents the difference between the total hemoglobin need and the amount that the single A gene can produce. In the disorders S-F and C-F a similar situation exists in that the amount of F hemoglobin produced represents the difference between the production of the single S or C gene and the total hemoglobin need. In all these instances, the failure of beta and delta chain production is completely compensated for by gamma chain production, and the total hemoglobin production is normal.

The mutation which produces persistent fetal hemoglobin therefore acts by interfering with the synthesis of two polypeptide chains, beta and delta chains of hemoglobin. This may be analogous to the deletions affecting the Lac locus of E. coli, recently described by Jacob *et al.* (24). They presented evidence that several distinct *structural genes* at the Lac locus were under the direction of another adjacent gene termed the *operator*. Certain mutations of the *operator* resulted in the absence of *several* products of the *structural genes*. The observations in the homozygous F individual who makes neither beta nor delta chains as a result of mutation might be an example of this mechanism.

In thalassemia major there is evidence that the defect in hemoglobin synthesis is not merely a lack of formation of normal hemoglobin, but that there is a derangement of hemoglobin production so that abnormal amounts of hemoglobin intermediates are formed (25, 26). Although these

products never enter the circulating hemoglobin pool, they contribute to the excretion of the degradation products of hemoglobin. Perhaps these hemoglobin intermediates interfere with the compensatory gamma chain production by blocking the recognition of the need for additional hemoglobin synthesis, and therefore in thalassemia major normal amounts of hemoglobin synthesis are not achieved.

SUMMARY

A family has been studied in which persistent fetal hemoglobin and beta chain thalassemia are present. Four individuals inherited both abnormalities (F-Thal) and demonstrated 67 to 70 per cent fetal hemoglobin and 30 to 33 per cent A hemoglobin. Their blood smears showed microcytosis, poikilocytosis, anisocytosis, and target cells, but no significant anemia was found. Another individual was homozygous for hemoglobin F (F-F) and at the age of twenty months had 100 per cent fetal hemoglobin. Although his blood smear showed marked red blood cell abnormalities, he had no significant anemia.

Twenty-two individuals who are heterozygous for F hemoglobin (A-F) have been studied. They have minor morphologic erythrocyte abnormalities, but no anemia or morbidity attributable to the abnormal hemoglobin was encountered.

Persistent fetal hemoglobin is inherited as an allele of the genes which affect the synthesis of the beta chain of the hemoglobin molecule. This mutation either specifically directs the synthesis of gamma chains of the hemoglobin molecule or prevents the production of normal beta and delta chains and permits compensatory synthesis of gamma chains.

The differences between hereditary persistence of fetal hemoglobin and thalassemia are discussed.

ACKNOWLEDGMENT

We are indebted to Miss Elisabeth Knüsel and Mrs. Mary Harry for valuable technical assistance.

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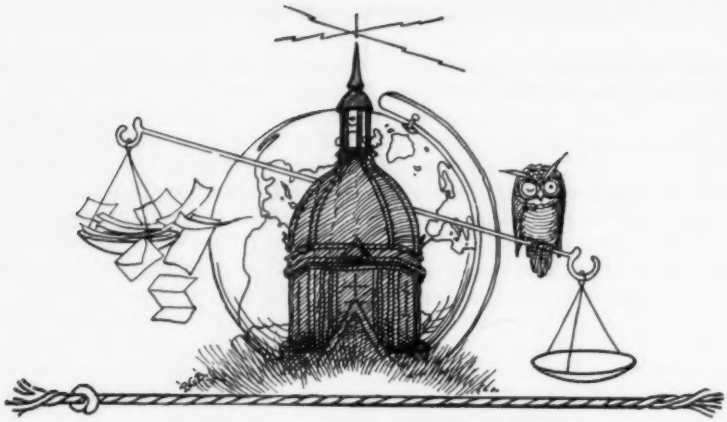

BOOK REVIEWS

Advances in Thyroid Research. Transactions of the Fourth International Goitre Conference. ROSALIND PITT-RIVERS, General Editor. 537 pp., $20.00. Pergamon Press, Oxford, England.

The fourth international conference on goiter took place in London in early July 1960 and was attended by about 600 physicians with forty-six countries represented. The meetings were held near the Houses of Parliament and Westminster Abbey. The building was of historic interest since the House of Commons sat in the attractive auditorium after the House was damaged by bombing during World War II. Those who attended, including this reviewer, were much impressed by the gracious hospitality of our London hosts. Moreover, the program was excellent; the scientific presentations as published numbered ninety-two and in addition there were three other addresses including the Dunhill Memorial Lecture by Sir Geoffrey Keynes.

The transactions make up, in a sense, a modern textbook on the thyroid. Although emphasis is placed on endemic goiter, there are significant contributions on neurogenic factors in thyrotoxicosis, on the prophylaxis, incidence and treatment of carcinoma of the thyroid, on the properties of various analogues of thyroxine, on immunochemical changes in thyroiditis, and on the nature of the binding of the thyroid hormones to the proteins of serum.

To recall the early work on endemic goiter by Dr. David Marine and Dr. Alan M. Chesney, both of whom are graduates of Johns Hopkins, adds interest to the reading of several chapters on this subject. Dr. Marine pointed out the role of iodine deficiency in the causation of endemic goiter and the prophylactic properties of iodine. Dr. Chesney and his associates noted goiters in rabbits fed raw cabbage; their observations on this type of goiter and its prevention by the administration of iodine were published in this Bulletin in 1928 in a series of three classic papers. Dr. Chesney has told me that he carried his quest for the identity of the goiter-forming substance in cabbage as far as the chemical knowledge of the day would allow him. The search for this substance or substances continues at present with significant progress, although the problem has not been entirely resolved. Greer and his associates of Portland, Oregon, isolated from raw turnips a "pro-goitrin" which after enzymatic hydrolysis becomes "goitrin". Goitrin is a thioglycoside of mustard oil which regularly induces goiter when fed to animals. Allcroft and Salt of Surrey in England, found that the goitrogenic properties of kale could be

radically altered by changes in fertilizer added to the soil during its growth. For example, the goitrogenicity of kale was increased when the soil was treated with Chilean nitrate of soda. On the other hand, its goitrogenicity was completely abolished by treating the soil with potassium iodate. Peltola from Finland found that milk obtained from the eastern sections of his country induced endemic goiter, but this property was not overcome by the addition of iodine. So, the nature of the goitrogens in some foodstuffs remains elusive.

In his memorial lecture Sir Geoffrey told of the interest which Sir Thomas Dunhill had in the thyroid gland. Dunhill was born in rural Australia and graduated from the University of Melbourne. He became rapidly an accomplished surgeon, but in his young days his accomplishments, especially in thyroid surgery, were little known outside of Australia. In 1907 he published in the *Intercolonial Journal of Australia* his experience of thyroidectomy in six patients and in 1908 in thirty-two patients with thyrotoxicosis. Dr. Halsted reported on his own technique in 1912 and at the time was unaware of Dr. Dunhill's accomplishments, for he made no reference to it. During World War I Dunhill served in Egypt and in France, following which he was persuaded to take up an academic position in London at St. Bartholomew's Hospital. His career was distinguished in many ways, not the least of which was his addition to the technique of surgery of the thyroid.

This book contains not only an interesting look at aspects of the developmental history of our knowledge of the thyroid, but also a section or two on all the more recent scientific accomplishments. Most of these sections are written lucidly and the scientific terms are simple and easy to follow. For this, the authors and editors deserve much credit. It is disappointing, however, that the bibliographies of several chapters are brief and indeed thirteen sections contain no references.

Sir Charles Harington pointed out in his presidential address that twenty-two years elapsed between the third and fourth international conferences on goiter. The success of this fourth meeting was so clear and the rapid advances in thyroidology of recent years so noteworthy that it is hoped the interval between the fourth and fifth conferences will be comparatively shorter.

SAMUEL P. ASPER, JR.

Virus Meningo-Encephalitis. Ciba Foundation Study Group No. 7. Editors for the Ciba Foundation, E. W. WOLSTENHOLME and MARGARET P. CAMERON. 120 pp., $2.50. Little, Brown & Co., Boston, Mass., 1960.

This short symposium type monograph expresses the views of a diversified group of virologists, neurologists, physicians interested in infectious disease and neuropathologists on viral meningo-encephalitides. The participants are cosmopolitan permitting an exchange of information on virus diseases of the nervous system between experts from different parts of the world.

A good share of this monograph is devoted to clinical and virological aspects of the effects of Coxsackie and "ECHO" viruses on the nervous system. There are shorter presentations of polio-meningo-encephalitis, tick-borne, "ARBO" virus and post-infectious encephalitides, and Behcet's disease. The discussions are lively, interesting and informative.

The reader of this monograph will be impressed with the identification of a growing number of viruses causing acute disease of the nervous system varying from mild to severe. The incrimination of viruses other than polio responsible for lower motor neuron paralysis must be increasingly suspect especially in those patients who have been adequately immunized against polio. Observations such as those reported here stress that

clinical observations provide no more than a hint to the correct virus diagnosis. Ultimately virological investigation is necessary for precise diagnosis. As shown by this study group, the judicious use of tissue culture, suckling mice and immunological techniques provides the necessary tools for virus isolation in an increasing number of diseases of the nervous system or viral origin.

This monograph is crisply written and contains much useful information. The material is up-to-date and relevant to those concerned with the clinical and laboratory diagnosis of virus diseases of the nervous system. The book is well worth the price.

CHARLES N. LUTTRELL

Essential Pathology. By ROGER D. BAKER. 638 pp., $9.50. The Williams & Wilkins Co., Baltimore, Md., 1961.

The author has attempted the difficult task of presenting both general and special pathology in a pocket-size volume. The book is sturdily bound and attractively produced. The selection of topics is generally sound. The only important exclusions noted were the omission of diabetic glomerulosclerosis and aleukemic leukemia. Illustrations are numerous and good. The gross photographs are particularly successful and the correlation of clinical histories with gross and microscopic illustrations is praiseworthy. The book is well written with a welcome absence of abbreviations and telegraphic phrases. Printing errors are very few. The emphasis on the frequency of the various diseases is unusual in pathology texts and is valuable.

Some statements are open to criticism. The phrase "histochemical lesion" appears objectionable to the reviewer. He also does not agree that human liver lacks regenerative powers and does not like the use of the word lymphoblast for glandular fever cells. The term adenomyosarcoma for Wilm's tumor is now only rarely used. The author's definition of cirrhosis omits regeneration which many pathologists and clinicians now consider essential. Hyalinization of splenic arterioles is not generally thought to be associated with hypertension.

Inevitably many topics are only briefly referred to in the text and the detail is insufficient for a medical student meeting the subject for the first time. The references suggested for further reading are, however, up-to-date and well selected.

BORIS H. RUEBNER

Essentials of Neurology. By JOHN N. WALTON. 422 pp., $6.75. J. B. Lippincott Co., Philadelphia, Pa., 1961.

This is a concise, well organized textbook of clinical neurology. It is well written, up-to-date and should fullfill the author's aim to provide the undergraduate student and practitioner with a composite picture of neurological illnesses. The chapters covering general principles and suggestions for investigating patients with neurological disease are clinically sound and cogent. Discussions on symptoms of neurological illness, disorders of the motor and sensory systems, developmental and degenerative disorders, infection and allergy, neoplasms and metabolic disorders are reasonably adequate albeit brief and sometimes a bit dogmatic. The final chapter is a useful outline of neurological treatment. A notable omission, however, was the recommendation for management of status epilepticus.

Apart from its brevity this book is little different from several other standard English texts on neurology. It will find its widest application among medical students and physicians requiring preparation for neurological questions in general medicine.

CHARLES N. LUTTRELL

Principles of Dynamic Psychiatry Including an Integrative Approach to Experimental and Clinical Psychology, 2nd edition. By JULES H. MASSERMAN. 332 pp., $8.00. W. B. Saunders Co., Philadelphia, Pa., 1961.

In his preface to the first edition (1946) of this small but ambitious volume, the author describes the difficulties of students entering the field of psychiatry who must be directed to many references scattered through an enormous literature in order to apprise themselves of the fundamentals of dynamic and clinical psychiatry. He has written this book with the objective of providing the neophyte with an orientative introduction in a single book. But more than that, the author attempts to integrate the various fields within or related to psychiatry (anthropology, psychoanalysis, psychobiology, communication theory, certain aspects of animal behavior and neurophysiology) among which there are "almost unbridgable semeiotic differences". In terms of both of these objectives, I think that the author has achieved some success and that in large measure the limitations of this book reflect the limitations of knowledge in the subsidiary fields of which psychiatry today is more of a patchwork composite than a logically coherent integration.

The first part of this book is entitled "Development of behavior theory". In it is presented an overview of "organic", psychologic, and psychoanalytic concepts of behavior. Part One concludes with two chapters devoted to neurotigenic and psychotic "dynamisms". In discussing claims made by the "organic school", the author rightly points up the lack of solid evidence in support of physiologic deviations that "are demonstrably the cause" of neurotic or psychotic behavior. Note is also taken of the false premises and lack of evidential support associated with the various "specific" physical therapies that have been in vogue at one time or another in psychiatry. In this connection, however, it is interesting that the author does not refer to the use of electroconvulsive therapy for severe depression which, in spite of its lack of theoretical basis or aesthetic attraction, appears to be significantly beneficial in a substantial proportion of cases. Likewise, pharmacotherapy is more or less dismissed by attributing the results, whether favorable or not, to "quasi-delusional" interchanges between psychiatrist and patient. There is no reference to conscientious efforts being made by a number of investigators to subject pharmacologic agents to careful, double-blind evaluation and such sweeping criticism points up the difference between scientific skepticism and *ex cathedra* condemnation.

Part Two is entitled "Biodynamics" and begins with a chapter in which is offered a critique of recent and past efforts in the areas of "Typology" of behavior, taxonomic psychiatry and various dynamic theories of behavior including Watson's behaviorism, psychoanalytic theory, Meyer's psychobiology, existentialism, and communication theory. In this chapter, the author's impressive erudition and his capacity to shift from an Olympian view to down-to-earth critical analysis are manifest. Incidentally, students who have had difficulty in comprehending the "precisely ambiguous" concepts of the currently popular existentialist movement will take comfort in the brief but pungently critical treatment of this topic.

In the next chapter, the author briefly outlines his well-known "general biodynamic theory of behavior". An important advantage of this theory is that the principles embodied in it are sufficiently broad and compatible with present day knowledge of biological adaptation as to provide a theoretical frame of reference useful in both clinical work and research in the behavioral sciences, human and animal. In the succeeding chapters (9 to 12), this applicability of the general biodynamic theory is vividly illustrated by descriptions of clinical anecdotal material and experiments with animals conducted in Masserman's laboratory.

The final two chapters are devoted to language, verbal and non-verbal, as related to

communication, adaptation, and therapy, and to the evolution of psychotherapy. Mention should also be made of the interesting and useful appendices among which is included a fascinating "illustrative psychoanalysis of a neurotic personality". In addition, there is a glossary of psychiatric terms and an extensive bibliography.

This book can be recommended not only to introduce newcomers to the field of psychiatry but also to any serious student engaged in work, clinical or research, in the behavioral sciences.

JOHN B. IMBODEN

Myasthenia Gravis. The Second International Symposium Proceedings. Edited by HENRY R. VIETS. 707 pp., $29.00. Charles C Thomas, Publisher, Springfield, Ill., 1961.

Two years after the Second International Symposium on Myasthenia Gravis was held in Los Angeles, a heterologous collection of papers has been issued and edited as the Proceedings of that Symposium. The various presentations cover a wide range of approaches to disorders of neuromuscular transmission, and themselves range in excellence from clear, concise, yet thorough treatises, to very poor disjointed ramblings. References (at the end of each chapter) generally reflect the individual author's work and interest, and figure-illustrations, to the point for the most part, in some instances are illegible and meaningless. Equal emphasis is given to untried, initial observations as to statistically valid and well-studied data. The indices seem complete, and division headings are understandable.

This expensive tome—or at least certain of the articles therein—can be recommended for occasional source material use.

MICHAEL P. MCQUILLEN

Annual Review of Pharmacology, Vol. 1, 1961. Edited by W. C. CUTTING. 479 pp., $7.00. Annual Reviews, Inc., Palo Alto, Cal.

This is the first volume for the Annual Review of Pharmacology. Those familiar with the Annual Reviews in other branches of medical sciences will recognize that the new series follows a familiar and successful pattern. The editors have selected a fine series of contributors and little criticism can be offered. While acknowledging the general excellence of the initial volume in a series, it is fair to warn that the editors may have a difficult task in preparing a second volume of equal quality. The present articles in general represent the most active and possibly the most interesting aspects of pharmacological research in recent years. It is difficult to imagine as interesting a table of contents next year, nor is it likely that the present subjects will maintain their present excellence if repeated at yearly intervals.

It seems unnecessary to mention the individual reviews in any detail. Suffice it so say that they maintain a uniform standard of excellence. Possibly the most original contribution is Leake's chapter entitled "Review of Reviews". Unfortunately it falls short of the intended mark. The author reviews the present problems involved in the documentation of scientific data, and briefly summarizes the developments in pharmacology and allied fields. In an attempt to provide an original approach to the bibliographical problem, Leake has contributed a chapter in which he briefly comments on approximately 100 review articles on pharmacological subjects that have appeared in the last three years. The major criticism is that the chapter lacks thoroughness and does not appear to be very systematic. The sub-headings are somewhat arbitrary and certainly do not represent the total field of pharmacology. Several reviews are obviously placed under the wrong heading. Unfortunately the bibliography of this chapter does not give the title of the article.

This would be of immense help in a chapter of this type. The major criticism is that one has little confidence that the review is complete. A quick perusal of only a few of the other chapters in the volume revealed sixteen articles that the reviewing authors chose to cite as important reviews in the past three years. Twelve of these are not cited in the chapter by Leake. These criticisms are offered with the belief that this approach may have something to offer if it is developed in a systematic and thorough manner.

The volume itself is a Review, the last chapter a Review of Reviews, and the present article has become a Review of Reviews of Reviews. Alas! Time to Stop.

GILBERT H. MUDGE

Arthritis and Allied Conditions. A Textbook of Rheumatology, 6th edition. Edited by JOSEPH LEE HOLLANDER, 1306 pages, $20.00. Lea and Febiger, Phila., Pa., 1960.

Interest in the rheumatic diseases continues to mount, and a vast amount of data is being recorded. It is in those areas of increased research activity that this edition has been primarily altered.

Included in the revised chapters are discussions of the rôle of immunology in the pathogenesis, clinical manifestations and diagnosis of the various rheumatic diseases. This supplements, in turn, the completely updated work on the systemic connective tissue disorders. Amyloidosis is discussed in the light of recent knowledge, as is the use of the adrenocorticoids, phenylbutazone and chloroquin in therapy.

Some of the new chapters are simply enlargements upon previous material and reflect the increased knowledge or importance of the particular subject since it was last written. Others are completely new to this edition, for example, those on the heritable disorders of the connective tissue and the syndrome of cervical nerve root compression.

Additions, both in the form of revisions or completely new subject matter, are presented in an informative manner. Once again the "box summaries," as Dr. Hollander calls them, are utilized to aid those who wish a quick, though incomplete, summary. The comprehensive reference source, on the other hand, should satisfy those who desire to delve further into a subject.

The editors have overcome the difficult task of writing a book which can function both as a textbook or a reference. It deserves the attention of anyone interested in arthritis and its related problems.

WILBUR J. BLECHMAN

The Rôle of the Physician in Environmental Pediatrics. By CARL C. FISCHER. 122 pp., $5.50. Landsberger Medical Books, Inc., New York, N.Y., 1960.

As stated in the preface, this book represents a collection of short talks offered to junior medical students which are designed to describe the rôle of the physician, particularly the pediatrician in comprehensive care. Dr. Fischer suggests that physicians fail to apply their skills to a large group of human problems. He selects five main categories; i.e., accident prevention, adoption, school health programs, the handicapped child and the adolescent, in which he examines the statistical incidence of problems peculiar to these areas and the rôle pediatricians play in these respective areas. The text is clearly presented on the level of the junior student. Apparently recognizing the difficulty in making these problems not only medical responsibilities but challenges to the prospective physician, Dr. Fischer has provided a collection of graphs and tables to illustrate the statistical data and support his personal plea for emphasis on "the era of social pediatrics." In chapter V the data on the incidence of handicaps are confusing and do not add up although the point is made.

Being a collection of separate talks the book is somewhat disjointed, however it is an

interesting distillation of a senior pediatrician's thoughts about the rôle of pediatrics in the midst of much controversey within the specialty itself.

ROBERT ALLYN KRAMER

BOOKS RECEIVED FOR REVIEW

An Atlas of Ophthalmic Surgery. By CONRAD BERENS AND JOHN HARRY KING, JR. 623 pp., $28.00. J. B. Lippincott Co., Philadelphia, Pa., 1961.

Basic Biochemistry. By M. W. NEIL. 360 pp., $6.75. J. B. Lippincott Co., Philadelphia, Pa., 1961.

Ciba Foundation Study Group No. 8. Problems of Pulmonary Circulation. Editors for the Ciba Foundation, A. V. S. DE REUCK AND MAEVE O'CONNOR. 96 pp., $2.50. Little, Brown & Co., Boston, Mass., 1961.

Clinical Haematology. By ROBERT DUNCAN EASTHAM. 158 pp., $4.00. John Wright & Sons Ltd, Bristol, 1961. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

Mayo Clinic Diet Manual, 3rd edition. By THE COMMITTEE ON DIETETICS OF THE MAYO CLINIC. 222 pp., $5.50. W. B. Saunders Co., Philadelphia, Pa., 1961.

The Nature of Sleep. Editors for the Ciba Foundation, G. E. W. WOLSTENHOLME AND MAEVE O'CONNOR. 416 pp., $10.00. Little, Brown & Co., Boston, Mass, 1961.

Neurophysiology. Reprinted from Medical Physiology and Biophysics. Eighteenth Edition of the Howell Textbook of Physiology. Edited by THEODORE C. RUCH AND JOHN F. FULTON. 403 pp., $10.00. W. B. Saunders Co., Philadelphia, Pa., 1961.

Pathologic Physiology. Mechanics of Disease, 3rd edition. Edited by WILLIAM A. SODEMAN. 1182 pp., $15.00. W. B. Saunders Co., Philadelphia, Pa., 1961.

Relief of Symptoms, 2nd edition. By WALTER MODELL. 374 pp., $11.50. The C. V. Mosby Co., St. Louis, Mo., 1961.

Textbook of Medicine, 13th edition. By Various Authors. Edited by SIR JOHN CONYBEARE AND W. N. MANN. 989 pp., $10.00. E. & S. Livingstone, Ltd., Edinburgh and London, 1961. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

Therapeutic Exercise, Vol. III, 2nd edition. Physcial Medicine Library. Edited by SIDNEY LICHT. 959 pp., $16.00. Elizabeth Licht, Publisher, New Haven, Conn., 1961.

Thoracic Diseases. Emphasizing Cardiopulmonary Relationships. By ELI H. RUBIN AND MORRIS RUBIN in association with GEORGE C. LEINER AND DORIS J. W. ESCHER. 968 pp., $25.00. W. B. Saunders Co., Philadelphia, Pa., 1961.

Varicose Veins. A Practical Manual, 3rd Edition. By R. ROWDEN FOOTE. 356 pp., $13.00. John Wright & Sons Ltd., 1960. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

Williams Obstetrics, 12th edition. By NICHOLSON J. EASTMAN AND LOUIS M. HELLMAN. 1230 pp., $16.00. Appleton-Century-Crofts, Inc., New York, N. Y., 1961.

THE BUERGER SYNDROME IN THE ORIENT

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In the writings of Leo Buerger and his successors a clinical picture which can be called *the Buerger syndrome* was developed. Its features are as follows: 1) Overwhelmingly predominant occurrence in males, 2) early onset (before 40 years in a majority of cases), 3) involvement of the upper extremities as well as the lower extremities in a significant proportion of cases, 4) predominent in arteries of intermediate and smaller size, 5) migratory thrombophlebitis in many cases, and 6) conspicuous relationship (of occurrence and aggravation) to tobacco smoking. In recent years it has been pointed out (1, 2, 3) that the diagnosis of "Buerger's disease" is much abused and that atherosclerosis and systemic embolism can produce the Buerger syndrome. Disease in the lower aorta and in the iliac arteries with thrombosis and/or embolism distally has also been proposed as a mechanism (11).

The point of uncertainty is whether there exist among the causes of the Buerger syndrome one or more distinct entities which can legitimately be called Buerger's disease. A second and at least partially independent question is, if there exists a distinct entity, Buerger's disease, is it a primary angiitis justifying the designation *thromboangiitis obliterans*? Wessler (4) concluded that "the disease originally described by Buerger is indistinguishable from atherosclerosis, systemic embolism, or peripheral arterial or venous thrombosis, singly or in combination." Fisher (1) studied "cerebral Buerger's disease" and questioned that the essential lesion is an angiitis. Thrombosis distal to obstruction in main arteries was present in the cases he studied. Reasoning by analogy, he wrote that "it is possible that peripheral Buerger's disease represents atherosclerotic occlusion of the aortic, iliac or femoral vessels associated with distal thrombosis due to slowed collateral blood flow with subsequent organization of the thrombus."

Gore and Burrows (2) reported postmortem examinations of three males, between 43 and 55 years of age, who in life were diagnosed as suffering from Buerger's disease. They concluded that the "so-called pathognomonic appearance of thromboangiitis obliterans is precisely that of an organized and recanalized thrombus in a muscular artery without intimal or other disease. . . . The basic lesion is arterial thrombosis which starts distally in small arteries and progresses by means of retrograde extension to the larger proximal channels. . . . Evidence of primary arterial inflammation was not

observed. Augmentation of the usual response to vasospastic stimuli is suggested as an explanation of the usual localization of the disease to the extremities." Thus, these authors did not deny the existence of a Buerger syndrome with special etiologic factors but rejected its inflammatory nature, which Buerger stressed.

A number of observations enjoin caution in rejecting out of hand the possibility that a specific Buerger's disease accounts for the Buerger syndrome in at least a small proportion of the cases. Some of these observations have been made in the West and have been referred to already by other writers (5, 6, 7, 8, 9) during the recent discussions. Here we will confine our attention mainly to observations on the Buerger syndrome in Korea and to some extent to those made in Japan. A serious difficulty with interpretation of the clinical and pathological aspects of the Buerger syndrome in the Occident is that any vessel-damaging process predisposes to atherosclerosis. By the time of necropsy, for example, atherosclerosis can be expected to have clouded considerably the fundamental process. Because of the lower frequency of atherosclerosis in the Orient less confusion in the interpretation of cases of the Buerger syndrome might be expected. The reports of a relatively high frequency of the Buerger syndrome in the Orient in association with a relatively low frequency of atherosclerotic and of thromboembolic disease suggested that the Buerger syndrome may be epidemiologically and therefore perhaps etiologically distinct. Such were the considerations which prompted the studies reported here.

METHODS AND MATERIALS

The principal part of the study was conducted at the Presbyterian Medical Center, a 140 bed general hospital located in Chonju, Korea. Chonju is a city of 100,000 population with a large surrounding agricultural area, the "rice basket" of Korea. Between January 1, 1957, and April 1, 1961, sixty-two new cases of the Buerger syndrome were admitted—sixteen in 1957, twelve in 1958, twelve in 1959, sixteen in 1960 and six in the first three months of 1961 (see Table I). (It is the impression of the staff (10) that rather numerous milder cases are seen as out-patients but not admitted for surgical treatment.)

TABLE I

Proportion of Buerger Syndrome in Hospital Admissions

Year	Total Inpatients	Buerger Syndrome	
		Number	Per Cent
1957	1,575	16	1.0
1958	2,044	12	0.6
1959	2,770	12	0.4
1960	1,562	16	1.0
1961	621	6	1.0

Letters were sent to these patients requesting them to come to the hospital for restudy. Twenty-seven responded to the letter, of whom five were first admitted in 1957, three in 1958, six in 1959, nine in 1960, and four in 1961. During the period of the study a twenty-eighth patient with Buerger's disease was admitted to the hospital with complicating tetanus.

With the assistance of the professional and technical staffs of the hospital, the twenty-eight patients were all investigated by the authors. The patients remained in the hospital or in inns in the vicinity, coming daily for investigation. In all patients a history was taken in the form of a questionnaire and examinations were performed according to a previously established outline. Narrative summaries are provided in Appendix I. Amputations, gangrene, ulceration, color changes and other visible changes were documented with color photographs. In addition, in all patients electrocardiogram and chest x-ray were made and fasting blood sugar concentration, serum cholesterol concentration, total serum protein, albumin and globulin, white blood cell count and hematocrit were determined. Fasting blood sugar was determined by the Folin-Wu method. Serum cholesterol was measured by the Bloor method (11). The values for level of serum cholesterol and blood sugar used in the analyses were the average of three separate determinations on the same specimen. In addition, frozen serum specimens were brought back to the Johns Hopkins Hospital where cholesterol levels were determined by a modified Bloor method (12). The agreement between the determinations made in Korea and those made in Baltimore was satisfactorily close. The methods gave comparable results (correlation coefficient 0.84). The values determined in Korea were systematically higher than those obtained in the United States, possibly because of loss through freezing and thawing in the latter case. Total protein, albumin and globulin were determined by the salting out method followed by nesslerization. Information on one or several urinary sugar determinations and on serologic tests for syphilis was already available. In many of the older patients x-rays for vessel calcification were taken of the pelvis and extremities. Femoral arteriograms, brachial arteriograms and aortograms were performed in selected patients to a total of twenty-four studies in fifteen patients. Despite limited capacity of the x-ray machine satisfactory contrast studies were obtained by use of extra-sensitive film.

Fresh material for histopathologic study was obtained from two patients: a limb amputated because of complicating tetanus infection (patient No. 26) and a biopsied nodule which was attached to the fascia of the right gastrocnemius muscle (patient No. 10). (Ten specimens from nine Korean patients with the Buerger syndrome hospitalized at the National Medical Center, Seoul, were also studied through the courtesy of Dr. J. Ringsted. See Section III below.)

Twenty-eight comparison subjects, all Korean males, were studied by all the same methods excluding arteriography. The age distribution (in years) of the study and comparison subjects is shown in Appendix II. Mean age was 38.4 years in the Buerger's group and 35.1 in the comparison group. Two of the comparison subjects were relatives who accompanied patients. One was a patient with Charcot-Marie-Tooth peroneal muscular atrophy, who had previously been thought incorrectly to have the Buerger syndrome. The remainder were Korean hospital employees and professional staff. Many of the hospital employees had been farmers previously and reported a diet essentially identical to that of the study cases (Table VI). Military and refugee history was quite similar. On the other hand, few of the hospital employees used tobacco or alcohol and current occupation was, of course, different. The current socioeconomic status of the twenty-five who were hospital personnel was undoubtedly higher. Because of the obvious differences between the study and comparison groups, reference to the second

group as a control has been intentionally avoided. A suitable control would probably be neighbors of the patients matched for age but such controls were not practical.

The hospital records of the thirty-four patients who did not return for restudy were reviewed. After the authors had left Chonju two of the thirty-four returned to the hospital and the same studies were conducted by members of the hospital staff who had participated in study of the twenty-eight patients. The results in these two cases are reviewed separately in Appendix I, where the cases are numbered 29 and 30.

As a frame of reference in which to interpret the experience with the Buerger syndrome, information was collected on other vascular disease in Korea and Japan: coronary artery disease, the aortic arch syndromes, arteriosclerotic abdominal aneurysm, the Leriche syndrome, phlebothrombosis and thrombophlebitis, pulmonary embolism and cerebrovascular disease. In Korea four other medical centers were visited—Seoul National University Hospital, National Medical Center (Scandinavian), Severance Medical College and Hospital and Soo Do Medical College, all in Seoul—and special inquiry was made on the frequency and clinical pattern of vascular disease.

In addition to the above experience six university medical centers in Japan were visited—Tokyo University, Juntendo University, Tokyo Medical and Dental University, and the Universities of Kyoto, Osaka and Nagoya. Particularly informative were visits to the service of Dr. Yoshio Hashimoto, professor of surgery at Nagoya University and that of Dr. K. Ishikawa, assistant professor of surgery at Tokyo University. Both have a long-standing interest in peripheral vascular disease and both have kept detailed records of their experience.

OBSERVATIONS

The findings of the study will be presented in four parts:

I. Clinical observations in Chonju, Korea.
II. Epidemiologic observations in Chonju, Korea.
III. Histopathologic observations in Seoul, Korea.
IV. Observations on the frequency of the Buerger syndrome in Japan.
V. Observations on the frequency of other vascular disease in Japan and Korea.

I. Clinical Observations

Sex: All twenty-eight patients studied in detail were male. The additional thirty-four patients whose records were reviewed were likewise all male.

Onset: As is seen in Table II onset was in the upper extremities in five of twenty-eight patients.

All but three of the twenty-eight patients had onset before 40 years and twenty of the twenty-eight before the age of 35 years (Table III). In almost a half (13/28) onset was before the age of 30 years. In two the disease started at the age of 14 and 16 years, respectively.

Interval between date of onset and time of study showed the distribution indicated in Figure 1, which also demonstrates that the longer the interval between the onset and the time of study the more extensive was the disease.

A somewhat indefinite dating of onset is suggested by a concentration of

TABLE II

Location of First Symptoms

Right leg	9
Left leg	12
Both legs	2
Right arm	2
Left arm	2
Both arms	1
	—
	28

TABLE III

Age at Onset

Before 15	1	30–34	7
15–19	1	35–39	5
20–24	5	40–44	2
25–29	6	45–49	1
			—
			28

TABLE IV

Extremities Involved

	Suggestive evidence	Rigorous criteria
4 extremities	15	11
2 legs, 1 arm	5	4
1 leg, 2 arms	0	1
1 leg, 1 arm	2	1
2 legs	3	7
1 leg	3	4
	—	—
	28	28

patients stating that onset was five years (eight patients), ten years (five patients) or fifteen years (two patients) before study.

Extremities involved: Considering all possible indications of involvement of the extremities the distribution of involvement in this series was as indicated in the left half of Table IV. In this evaluation symptoms of coldness or paresthesias in the hands and suspiciously abnormal findings on palpation of the pulses and Allen test (13) were included, as well as more definite change such as gangrene of fingers and absent radial or antecubital pulse. Failure to palpate the ulnar pulse and slow filling of the hand via the ulnar artery on Allen test cannot alone be taken as indubitable evidence of disease. If questionable cases are excluded and only those

with absent radial pulse or unquestionably feeble radial pulse are taken as having arm involvement, the distribution becomes as indicated in the right half of Table IV.

Thus it is seen that seventeen of twenty-eight cases had indubitable involvement of one or both arms in addition to leg involvement. Some of the other cases, such as those classified as showing suggestive signs, almost certainly had arm involvement. From this standpoint two patients were of interest: their first symptoms were coldness and color change in one finger. These disappeared spontaneously with no objective signs in the arms at the time of examination.

In eleven of twenty-eight patients one radial pulse was totally impalpable and in one additional patient neither radial pulse could be felt. Cervical sympathectomy had been performed in five patients (unilateral in Nos. 4, 10 and 12; bilateral in Nos. 13 and 18).

Rigorous criteria of leg involvement were absence of both foot pulses, or amputation of at least toes, singly or in combination. Symptoms of claudication, numbness or coldness of the leg or foot were taken as suggestive evidence in assembling the data shown in Table IV.

Level of involvement in the extremities: One patient had absent brachial pulse in one arm; the axillary pulse was palpable. The patient (No. 12) had onset of long persisting ulceration in the right great toe fifteen years before study and symptoms beginning in the arms five years after onset. Three years before study symptoms began in the left leg. There was no history of frostbite and no history of trauma except that which supposedly initiated the ulceration. The clinical picture is that of a progressive disease involving in time all four extremities and involving progressively more proximal arteries, e.g., the antecubital and brachial arteries, with pulse remaining in the axillary artery.

Two patients (Nos. 1 and 17) had absent femoral pulses bilaterally and three (Nos. 6, 11 and 16) had bruit and thrill over the right femoral artery. One patient (No. 12) had an absent right femoral pulse.

Veins: Three of the patients with Buerger syndrome (Nos. 10, 20 and 25) had mild varicosities. Four of the comparison subjects (Nos. 32, 34, 35, 41) had varicosities of about the same proportions. It is impossible, therefore, to attribute any significance to the findings in the Buerger's patients. Because of difficulties of interpretation no special attempt was made to get a history relating to thrombophlebitis. However, one patient (No. 2) described as the initial symptom of his disease the development of a painful, red, hot, tender mass on the medial aspect of the right lower leg. Thrombophlebitis in this series of Oriental patients with the Buerger syndrome may indeed be rarer than in Occidental patients, as suggested by Snapper (14); however, no firm conclusion on the basis of this survey is justified.

Without sympathectomy the lack of normal fullness of the veins in the involved extremities paralleled rather closely the other evidence of impaired circulation. A pronounced difference was noted between the Buerger syndrome series and the comparison series as to the ease with which venous blood was drawn from the arm for laboratory tests. Most of the Buerger syndrome patients had arm involvement; the reduced arterial flow in the arms had its natural counterpart in reduced venous flow.

Other physical findings: No xanthelasmata or other xanthomata were discovered in any subject of either group. No arcus senilis was found in the study cases. One of the comparison cases (No. 49, age 45) had arcus senilis.

No incidental disease of particular note was discovered in the study cases. In the comparison cases mitral regurgitation was discovered in one (No. 48) and retinitis pigmentosa in one (No. 41).

The cardiac examination revealed no abnormality in any study case.

Contrast studies: Most of the arteriograms revealed obstruction of leg vessels in the thigh or upper calf area and of arm vessels at or just below the antecubital fossa. With rare exception (e.g., Fig. 12) no change suggesting atheroma was seen. The arteries looked smoothly lined and regular in caliber, sometimes with gradual tapering, sometimes with abrupt interruption.

In the arm a frequent finding was persistence, and in many, dilatation of the interosseous artery with occlusion of the radial and/or ulnar arteries.

Other laboratory findings: No instance of hyperglycemia or glycosuria was discovered. The mean values for fasting blood sugar were 93.1 mg. per cent (S.D. 9.1) for the Buerger syndrome and 91.3 mg. per cent (S.D. 5.3) for the comparison cases. (In one patient, No. 29, not included in the personally studied series, mild hyperglycemia and hypercholesterolemia were found. Diabetes may be a contributing factor in the otherwise typical four-extremity Buerger syndromes in this patient.)

There was no instance of positive serologic test for syphilis. Chest x-rays showed cardiac enlargement in no instance. No calcification of the aortic knob, pelvic vessels or vessels of the extremity was discovered in any patient. No abnormality of the electrocardiogram was discovered in either the patients or the comparison group. One member of the comparison group (No. 34) had right bundle branch block, presumably as a normal variation.

The cholesterol data are recorded in Appendix II. There was no significant difference in the mean values (196.8 mg. per cent, S.D. 20.3, for the Buerger syndrome; 201.3, S.D. 27.2, for the comparison group). Furthermore the regression of cholesterol on age was similar ($F = 1.192$; $p < 0.3$) and age adjusted means were also similar ($F = 0.697$; $p < 0.5$). The

age and cholesterol values had comparable means and scatters in the two groups. The variance of cholesterol level in the comparison group was greater than that in the Buerger's group due possibly to greater variance in diet in the former group. The values were higher than those in Koreans reported by others (15) but differences in method of determination are undoubtedly responsible. The Bloor method gives, it is well known, higher values than many others.

The serum cholesterol levels determined in Baltimore on the same specimens brought from Korea in a frozen state showed mean values of 170.4 mg. per cent (S.D. 29.3) for the study group and 180.8 mg. per cent (S.D. 31.5) for the comparison group. (Serum cholesterol values determined in the two laboratories showed a correlation coefficient of 0.84; the values as determined in Baltimore were systematically lower, due possibly to losses in freezing and thawing.) Assuming that the values as determined in Baltimore are representative of the true values in the groups of subjects studied, then only 5 per cent of an American white male hospital population, age 40 to 59, in which the determinations were done by the same method and in which no evidence of diabetes mellitus or disease of the thyroid, liver or kidney was present (12), had a serum cholesterol as low or lower than the mean of the cases of Buerger's disease. Hypocholesterolemia in the Korean subjects relative to subjects in the United States is demonstrated.

In histopathologic material from two (Nos. 19 and 26) of the twenty-eight patients with the Buerger syndrome no changes indicative of a primary angiitis could be detected.

II. Epidemiologic Observations

The parent population: The twenty-eight cases were drawn from sixty-four patients who were admitted to the Chonju hospital between January 1957 and March 1961 and in whom the diagnosis of Buerger's disease was proposed. In two the diagnosis was found to be in error. One, a 40 year old woman with gangrene of one finger, was later proved to have leprosy. One patient, a male age 24, with complaints of weak legs and cold feet, had clear evidence of Charcot-Marie-Tooth peroneal muscular atrophy. The remaining sixty-two cases of the Buerger syndrome were all male. What relation does the series of twenty-eight study patients bear to the total series of sixty-two patients from which they were drawn? The twenty-eight cases which were self-selected for close study tended to be cases admitted to the hospital in more recent years (see Fig. 2). This may in part be due to greater facility of contact by mail, that is, less change of address, in more recent cases; in part due to greater persisting rapport between hospital and patient in recently seen patients.

TABLE V

Extent of Involvement as Recorded in Hospital Record of 34 Patients Who Were Not Studied by the Authors

4 extremities	1/34
2 legs, 1 arm	5/34
1 leg, 1 arm	1/34
2 legs	14/34
1 leg	13/34

As seen in Table V, as compared with Table IV, the extent of involvement recorded in the hospital records of the thirty-four patients not actually examined was much less than in the twenty-eight study patients. In part the difference is not real; the extent of involvement as indicated in the hospital records is doubtless an underestimate because of failure in many instances to record specific information on the state of the pulses, the results of Allen tests and the occurrence of symptoms in the hands. In addition, however, it seems likely that the study group indeed had more severe and extensive involvement. A patient with more severe disease would be expected to respond more readily to an invitation to return for study. Furthermore, on their return for follow-up, they were *ipso facto* later in their disease, on the average, than the group as a whole when seen at first admission. There is a demonstrable relationship between duration of disease and extent of disease (Fig. 1). These explanations are supported by a comparison of extent of involvement recorded in the hospital records of the study patients with the extent of involvement determined in this investigation.

The total number of admissions to the Presbyterian Medical Center in each year is indicated in Table I. The total experience with the Buerger syndrome is not represented by the number of admissions of patients with that diagnosis. The number of out-patients seen in a year is approximately ten times the number of admissions. Although no diagnostic indexing of these patients is available, it is the impression of the staff that the Buerger syndrome is encountered rather frequently, that only the most severe cases are admitted, and that the total experience involves several times as many patients as are admitted for surgical treatment.

Some indication of the larger population from which the sixty-two cases of the Buerger syndrome were drawn is provided by the map in Figure 3. As indicated below there was probably an increased proportion of rural dwellers.

Familial aggregation: Two brothers were represented in the series, cases 20 and 21. No evidence of vascular disease was discovered in a third asymptomatic brother who was also examined.

Unlike Japan, consanguineous marriage is exceedingly uncommon in

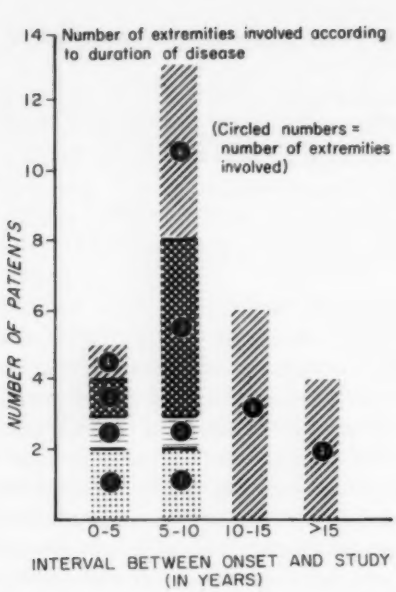

FIG. 1. The involvement tended to be more extensive when the disease was of longer duration.

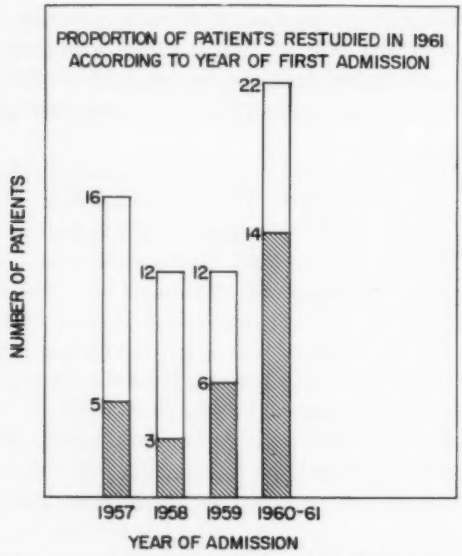

FIG. 2. The rate of response for follow-up tended to be higher when the first admission to the Presbyterian Medical Center was more recent. The differences are significant at a level between 5 and 10 per cent. Probably an analysis according to date of last contact with the PMC would have yielded more striking differences.

FIG. 3. The distribution of residence of the sixty-two patients with the Buerger syndrome from whom the study group was drawn. Each point refers to a single case. The number of cases from each of five cities is indicated with the name of the city. Except for those seven patients who lived in the cities specifically named on the map the patients all lived in farm villages. Note that six lived on islands off the southern coast.

Korea (16). Even marriage of persons of the same name is avoided. A relatively small number of frequently occurring names accounts for most Koreans; in this series of fifty-six study and comparison subjects, for example, the name Kim occurred ten times, Pak (or Park) five times, and Cho, Chung, Lee, and Yue four times each. There were among fifty-six patients and controls only twenty-five different names. The restriction, even on marriage of persons of the same name, guarantees a low rate of consanguinity.

Of the fifty-four parents of twenty-seven study cases, thirty-one were still living; for the twenty-seven comparison group in which the information was obtained the figure was also thirty-one. Usually the informant was not familiar with the cause of death of his parents.

Occupation and socioeconomic status: All but five of the twenty-eight patients were farmers or farm laborers, living in small farm villages (Fig. 3). All twenty-three were of the lowest socioeconomic status. The five non-farmers were (or had been) machine operator in a rice mill (No. 5), government clerk (No. 18), clerk and foreman in a construction company (No. 19), operator of a lumber shop (No. 28) and a seamen-fisherman (No. 10).

That the proportion of rural dwellers, and therefore of farmers and laborers, was indeed greater than one could expect from the origin of the general patient population is suggested by the map in Figure 3. Although about 20 per cent of inpatients came from the city of Chonju, only two of sixty-two patients in the larger cities came from Chonju. Six of the larger group of sixty-two and two of the study group of twenty-eight lived on islands off the southern coast.

Indirectly the increased frequency of the Buerger syndrome in farmers is suggested by a comparison of the frequency of the disease in the Chonju Hospital and in the Severance Hospital, Seoul. The Severance Hospital admitted several times as many patients in the period from 1957 to 1961 but admitted only about half as many cases of the Buerger syndrome. Seoul with a current population in excess of two million contributes a majority of the patients of the Severance Hospital. Fewer farmers and farm laborers are admitted to Severance Hospital than to the Chonju Hospital.

Exposure: South Korea lies between the thirty-fourth and thirty-eighth parallels—approximately the latitudes of Columbia, S. C., and Washington, D. C. Extremes of temperature in the Chonju area are about 10° and 100° F. The climate of Chonju and its environs is almost identical to that of Baltimore, i.e., Korea is colder than the part of the United States at the corresponding latitudes.

The Korean farmer works long hours, much of it with feet and hands in the cold waters of the rice paddy. The rubber shoes (Figs. 8A, 17A) customarily worn by the Koreans afford little protection against cold, especially when water gets inside. Houses are ill heated. Fuel is scarce, especially since the mountain sides have been largely denuded by war and wasteful cutting. South Korea's coal, scarce and of poor quality, is not within the means of the farmer.

Six of the patients and one of the comparison cases suffered frostbite of the extremities. It was difficult in the patients to be certain whether the frostbite antedated the circulatory impairment or followed it as a consequence. In some there is room for suspicion that frostbite was a factor precipitating progressive vascular disease. In others it seemed more likely that frostbite was a symptom of circulatory impairment.

No relationship could be established between season of year and either onset of symptoms or date of first admission to the hospital.

Frostbite *per se* has a clinical picture quite different from the Buerger syndrome (17, 18). Although frostbite might be a complicating factor and perhaps one of several initiating factors, the disease in these patients is not purely frostbite.

Cold hemagglutinins were not studied. Cold hemagglutinating tendency shows a racial difference (19). The United States Negro shows significantly higher titers and a higher frequency of the phenomenon than American whites. Mongolians have much higher titers than either whites or Negroes.

Trauma: Of twenty-seven patients, at least eleven had suffered trauma to the extremities. For example, patient No. 15 lost his right index finger and had multiple shrapnel lodge in the left forearm in 1953 during the Korean War. The Buerger syndrome, which subsequently involved also both legs, began in the left arm in 1956, although he noted loss of his left radial pulse immediately after the shrapnel wound (See Fig. 13). Patient No. 3 had an abscess of the left calf muscle which drained spontaneously in 1941 and had onset of the Buerger syndrome in the left leg in 1958 with subsequent involvement of the right leg and possibly the right arm. Patient No. 19 suffered a through-and-through bullet wound of the left calf in 1950; the Buerger syndrome began in the left leg about two years later with subsequent involvement of the right leg and right arm.

As in the case of frostbite, it is difficult in some instances to be certain which came first, trauma or vascular insufficiency. For example, patient No. 5 burned his feet at the onset of his illness, which progressed to involve all four extremities. Patient No. 12 sustained an injury to the right great toe by a sharp object and thereafter had a long persisting ulceration; involvement of all four extremities developed. Further difficulties of interpretation result from the fact that the population from which the cases were drawn is exposed to unusual trauma because of occupation and the unsettled political circumstances of the last two decades.

In three of twenty-eight there was a history of trauma specifically to the sacral area and in two of these three patients disease was located at a high level. Patient No. 16 was struck in the sacral area while in jail about 1945. Symptoms of circulatory insufficiency began in the left leg in 1956 when the patient was 35 years old. The right leg has subsequently become involved. The arms are by all evidence not involved. The right femoral pulse was weak and associated with a prominent systolic bruit and thrill. Popliteal and foot pulses were felt in neither leg. Arteriograms showed that the right external iliac artery was markedly narrowed and there was a long narrowed segment of the left femoral artery (Fig. 14). Post-stenotic dilatation was present bilaterally.

Patient No. 22 was struck in the sacral area by a communist about 1951.

Symptoms of the Buerger syndrome began in the left leg in 1960. No other extremities have been involved. Left lumbar sympathectomy was performed in late 1960. No pulse deficit whatever was detectable in 1961 and only mild claudication in the left leg persisted.

Patient No. 17 was struck many blows in the low back, sacrum and legs about the knees about 1946. Symptoms began about five years later with pain first in the left inguinal area and left thigh and later in the right leg. About 1956 he felt tender nodules in the veins of both lower legs suggesting thrombophlebitis. Bilateral lumbar sympathectomy and right above-knee amputation were done August 1960, and left below-knee amputation in January 1961. Examination in 1961 revealed no femoral pulses. Aortogram revealed complete obstruction of the left iliac artery at its origin from the aorta and the right iliac was markedly narrowed at the same level (Fig. 15).

It is felt that cases 16 and 17 and possibly also 22 represent a different condition from that present in the majority of the patients in this series. It is noteworthy that arm involvement was not present in any of the three. The occlusive arterial disease may be related to the trauma. The aortograms do not suggest atherosclerosis. The cases may be similar to those reported by Boyd and Jepson (20); external iliac artery thrombosis occurred in a 28-year-old man after being struck across the lower abdomen and thigh by a large branch of a falling tree and in a 23-year-old sprint cyclist.

Tobacco: All patients were smokers. In two of the twenty-eight patients the onset of disease may have antedated smoking but this is not certain. Cigarettes or the long Korean pipes, mainly the former, were smoked. Deep inhalation was the rule. The smoker usually relighted butts or smoked cigarettes to the very end. Many used cigarette holders to permit complete smoking. The tobacco smoked was mostly Korean, which is ranker than that in American cigarettes.

Many of the patients stopped smoking for a few weeks or months after admission to the Presbyterian Medical Center and some of these apparently had relief of symptoms during this period. Since surgical therapy was also given the influence of abstinence from tobacco is hard to evaluate. One patient (No. 23) who stopped smoking completely three years previously had apparent arrest of the disorder and still worked as a farmer despite signs of four extremity involvement and a "below knee" amputation on the right.

Complete relief of symptoms with cessation of smoking cannot be expected since vascular occlusion persists. Progression of the disease as indicated by worsening of symptoms and the development of new ulceration and gangrene can be the only basis for judging the effect of smoking and non-smoking.

Very few Korean women smoke.

Alcoholism: Many of the patients consumed large amounts of alcohol. Rice wine (sul) and to a lesser extent a whisky-like liquor distilled from rice (soju) are drunk. Alcoholism might be a factor contributing to the progress of the disease by leading to unusual exposure during drunken states, but this can be only speculation.

Diet: The staples of the Korean diet are rice, soya sauce, soya bean cake and kimchi. The last is pickled cabbage and other vegetables intensely seasoned by red and black pepper, ginger and other condiments. The diet is relatively high in salt since much is contained in both soya sauce and kimchi.

Dairy products are completely absent and eggs are almost totally lacking from the Korean diet. Little meat and fish is eaten (Table VI). A few patients had eaten eggs "for strengthening" *only* after the onset of their present illness. Eggs are usually eaten raw by sucking the contents through a small hole made in the shell at one end. The frequency distribution of fish and meat consumption was as indicated in Table VI. Clearly the diet of these patients is not that which is considered to be atherogenic. What fat is consumed is derived largely from vegetable sources (Table VII).

The diet of comparison subjects is also shown in Table VI.

TABLE VI

Times per year	Meat	Fish	Eggs
A. Diet in Study Cases			
0	1	2	Essentially 0 in all (see text)
<6	11	7	
6 > 12	5	2	
12 > 24	6	7	
24 > 52	4	5	
52 > 72	—	4	
Daily	1	1	
	28	28	
B. Diet in Comparison Cases			
0	1	1	11
<6	1	1	—
6 > 12	—	3	—
12 > 24	7	6	1
24 > 52	9	10	3
52 > 72	2	3	2
72 > 150	7	4	3
Daily	1	—	8
	28	28	28

TABLE VII

Korean Farmer's Diet

(Adapted from table assembled by Young-Choon Lee, M.D., Director, The Institute for Rural Health, Kaejong, Korea, using data from Dr. Jung-Duk Song, Biochemistry Department, Yonsei University, Seoul, and the Korean Food-Analysis Tables of the National Institute of Chemistry, Seoul.)

	Spring	Summer	Autumn	Winter
Food intake (gms.)	*885*	*1931*	*1377*	*1104*
Vegetable origin	869	1839	1349	1068
Animal origin	16	92	28	36
Fat (gms.)	*12.0*	*28.4*	*51.8*	*13.0*
Vegetable origin	10.2	17.8	49.6	10.3
Animal origin	1.8	10.6	2.2	2.7
Protein (gms.)	*60.4*	*103.5*	*144.7*	*69.8*
Vegetable origin	58.8	83.9	137.9	58.2
Animal origin	1.6	19.6	6.8	11.6
Carbohydrate (gms.)	*494.9*	*542.7*	*520.5*	*505.6*
Vegetable origin	494.9	541.8	520.3	505.3
Animal origin	—	0.9	0.2	0.3
Food intake (cal.)	*2329.2*	*2840.4*	*3127.0*	*2418.6*
Vegetable origin	2221.2	2584.8	2660.8	2301.6
Animal origin	108.0	255.6	466.2	117.0

Shown in Table VII is a break-down on the average Korean farmer's diet. The food harvested in the fall (rice and kimchi) are barely adequate for the farmer's sustinence during the winter period. A spring famine of some proportion, often severe, occurs annually before the barley is ready for harvest.

As shown in the map (Fig. 3) six of the sixty-two patients lived on islands off the southern coast of Korea. The study group of twenty-eight patients included two island residents. The diet in the islands includes more fish, and barley, which grows better there, is substituted for rice to a considerable extent.

In the dietary history no attempt was made to determine consumption of grains which might be ergoty. Lower socioeconomic classes eat rice which is less highly polished and cleaned than that enjoyed by their more favored countrymen (10). Furthermore, barley is substituted for rice by poorer people. Barley is harvested from the rice paddies in the late spring before the rice seedlings are set out. The marginal farmer eats much barley between the barley harvest and the rice harvest (10).

Constitution: No pecularities of constitution impressed the observers. Most of the patients had sparse body hair everywhere, for example, on the chest. Sparsity of hair, frequently total lack of hair, on the lower legs was difficult to evaluate in terms of trophic change. Patients often

stated that they never had hair on the legs, that is, that there had been no change.

III. Histopathologic Observations

Histopathologic material from ten amputation specimens in nine Korean male patients was reviewed through the courtesy of Dr. J. Ringsted of the National Medical Center, Seoul. The ages of the nine patients were 25, 25, 31, 35, 39, 40, 41, 45 and 52 years. The clinical diagnosis was "Buerger's disease" in all. In all organizing or organized, recanalizing or recanalized occlusion was present. In two cases destructive changes in the media, particularly the internal elastic lamella, suggested a previous inflammatory change. In one the entire wall of several arteries as well as the organized thrombus of the lumen was involved in a subacute inflammatory process and the internal elastic lamella showed multiple fractures (Fig. 4). The patient, age 25 years, had "below knee" amputation of the right leg for gangrene of the toes and foot. The vessels studied were thought to be remote from the area of actual necrosis and possible infection.

IV. Observations on the Frequency of the Buerger Syndrome in Japan

The Japanese literature (see review in Tables VIII, IX, X and XI) supports the impression of a relatively high frequency of the Buerger syndrome[1] (26). We learned on our recent visits in Japan that between five and twenty-five cases are admitted each year to university surgical services. One can assume that in the reported cases summarized in the tables diabetes mellitus, hypertension, hyperlipidemia and heart disease were not present. The great majority were male and 62 per cent were less than 40 years of age at the time of first hospital visit.

There are several possible explanations for the lower proportion of upper extremity involvement in the assemblage of Japanese reports (Table XI) than in the Chonju study: 1) In the Japanese series the patients were undoubtedly earlier in their disease (since their condition at first admission was recorded) than the patients recalled for follow-up at Chonju. 2) Japanese patients may seek medical attention earlier than Korean patients. 3) The criteria for upper extremity involvement may have been quite different.

Koyano (25), who reported on 120 cases occurring between 1900 and 1921, pointed out that they 1) were recruited almost exclusively from the laboring class, 2) were mostly males age 20 to 40 years, 3) often suffered exposure of the hands and feet to wet and cold, in work in the rice paddies, for example, and finally 4) were almost all smokers.

[1] Early reports of thromboangiitis obliterans in Japanese were those of Haga (21) in 1898, Ito (22) in 1910, Todyo (23) in 1912, Koga (24) in 1913, and Koyano (25) in 1922.

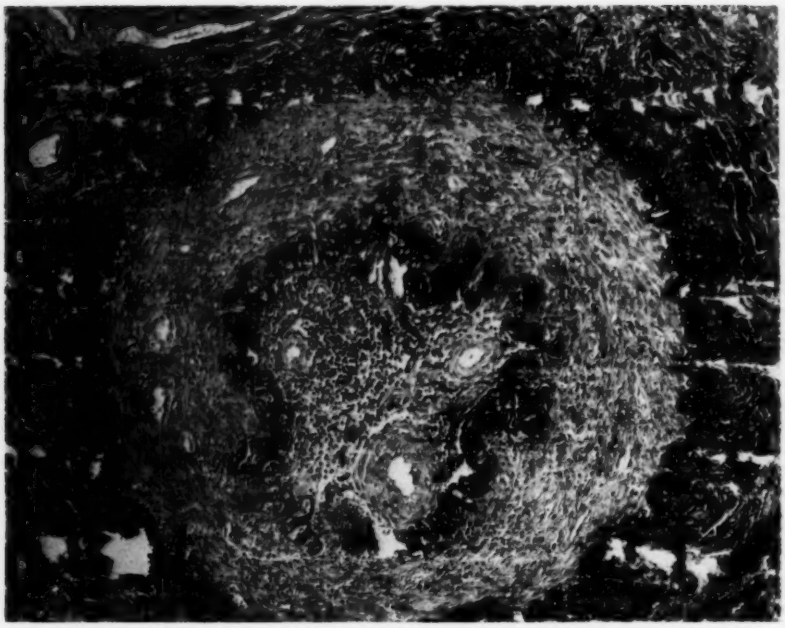

FIG. 4A.

FIG. 4. Histopathologic observations in a 25-year-old Korean male with the Buerger syndrome necessitating amputation of the leg. A, B. Views of arteries showing inflammatory cells through the wall, recanalizing occlusion of the lumen, inflammatory change in the occluding tissue and destruction of the internal elastic lamella at some points. Verhoeff stain, ×100.

V. Observations on the Frequency of Other Vascular Disease in Japan and Korea

The frequency in the Orient of other cardiovascular disease, especially that of atheromatous nature, is highly pertinent to the interpretation of the cases of the Buerger syndrome. In brief, it can be stated (27, 28, 29, 29a) that coronary artery disease is exceedingly rare, especially in the socioeconomic group which is affected by the Buerger syndrome. Hypertension is frequent, probably at least as frequent as in the West (29). Cerebrovascular disease, probably related to the high frequency of hypertension,[2] is the most frequent cause of death in Japan (30). It is also a frequent cause of death in adults in Korea, being surpassed only by tuberculosis. Diabetic gangrene is almost completely unknown. Maekawa (31) stated that from

[2] Probably mainly cerebral hemorrhage but also cerebral thrombosis on the basis of hypertensive arteriopathy.

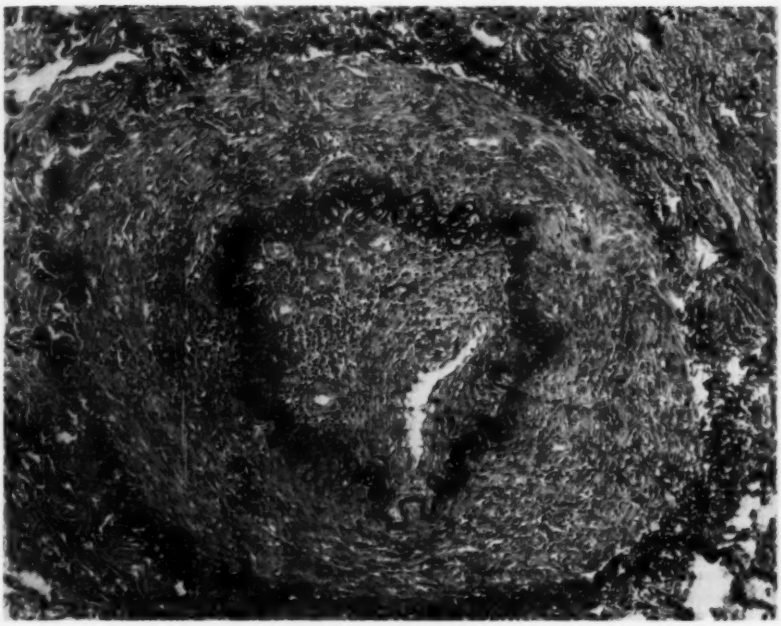

FIG. 4B.

TABLE VIII

Sex and Incidence of the Buerger Syndrome
(Selected Japanese Reports)

Reporter	Year	Male	Female	Total
1. Karatsu (38)	1935	35	5	40
2. Kawaguchi (61)	1935	58	2	60
3. Kumamoto (57)	1940	117	8	125
4. Saito (58)	1950	253	16	269
5. Urakubo (59)	1954	94	18	112
6. Kawata (60)	1954	124	19	143
7. Ishikawa (26)	1954	223	9	232
Total		904 (92.1%)	77	981

1950 to 1955, sixty-four cases of gangrene of the extremities were admitted to the surgical service of the Kyoto University Hospital. Of these, fifty-nine had what was diagnosed Buerger's disease and only two had diabetic gangrene.

Phlebothrombosis and thrombophlebitis are rare. Pulmonary embolism,

TABLE IX

Age of Presentation in the Buerger Syndrome

(Selected Japanese Reports)

Reporter	Age (years)								
	<10	<20	<30	<40	<50	<60	<70	<80	Total
1. Karatsu (38)		1	11	13	6	8	1		40
2. Kawaguchi (61)			4	14	18	2			38
3. Kumamoto (57)	1	7	43	38	28	6	2		125
4. Saito (58)		3	40	97	93	23	12	1	269
6. Kawata (60)		3	24	40	37	21	18		143
7. Ishikawa (26)		3	72	98	39				212
Total	1	17	194	300	221	60	33	1	827

TABLE X

Localization of Affected Extremities

(Selected Japanese Reports)

Reporter	Upper Extremity	Lower Extremity	Total	No. of Patients
1. Karatsu (38)	8	41	49	40
2. Kumamoto (57)	43	173	216	125
4. Saito (58)	108	328	436	269
6. Kawata (60)	26	178	204	143
7. Ishikawa (26)	70	307	377	232
Total	255	1027	1282	809

TABLE XI

Number of Affected Extremities

(Selected Japanese Reports)

Reporter	1 extremity		2 extremities			3 extremities		4 extremities	Total
	1 hand	1 foot	2 hands	2 feet	1 hand 1 foot	1 hand 2 feet	2 hands 1 foot	2 hands 2 feet	
Karatsu (38)	2	21	2	14				1	40
Dumamoto (57)	7	55	4	45				14	125
Saito (58)	19	136	8	59	13	12	4	28	269
Kawata (60)	6	87	3	38	3	1		5	143
Ishikawa (26)	8	101	1	46	18	21	4	25	224
Total	42	400	18	202	34	34	8	63	801
Total (by number of extremities)	442		254			42		63	
Per cent	55.2		31.7			5.2		7.9	

for example after surgical operations, is much rarer (32) than in the United States.

Thromboembolic complications are thought to occur much more rarely in Japanese and Korean patients with mitral valvular disease and with myocardial infarction.

Arteriosclerotic abdominal aneurysm is occasionally encountered (33) but may be rarer than in the West. One form of vascular disease which is distinctly more frequent in the Orient than the West is the giant cell arteritis and aortitis which affects young females and leads to so-called pulseless disease (Takayasu's disease; "young female arteritis" type of aortic arch syndrome). Takayasu's disease is an undisputed entity and is clearly distinct from "Buerger's disease" (34).

One case of the Leriche syndrome (thrombotic obliteration of the bifurcation of the aorta) reported from Japan (35) occurred in a patient with diabetes mellitus. Ishakawa (36) observed three cases among 273 of peripheral vascular disease. There are at least two other case reports of Leriche syndrome from Japan (37, 38). Furthermore, by personal communication it is learned that Inoguchi of Fukuoka City saw one case in 1959 and two in 1960 (39).

Autopsy experience on Korea: The National Medical Center (Scandinavian) is Seoul probably has the most extensive recent autopsy experience in Korea. Koreans are reluctant to permit postmortem examinations. Ringsted (40) has recently reviewed the experience in 342 autopsies (excluding those on stillborn babies and a few westerners) performed since the opening of the hospital in December, 1958. The autopsies represented slightly more than 90 per cent of deaths in the hospital. Infectious disease and malignancies represented the largest proportion of cases, 21.1 and 22.1 per cent respectively. In *no* case was arteriosclerotic and degenerative heart disease discovered as the cause of death. All cases of vascular lesions of the central nervous system were either ruptured congenital aneurysm or cerebral hemorrhage occurring in hypertensive patients. The degree of atherosclerosis in the aorta was notably low. Recently, the degree has been graded with the method of Holman with results which to date appear to corroborate the previous impression.

Pulmonary embolism and venous thrombosis were observed in 4.7 per cent of all autopsies—a very low figure compared with the experience at the autopsy table in western countries. In no instance was death attributed to pulmonary embolism.

DISCUSSION

Other indications of a relatively high frequency of the Buerger syndrome in the Orient: In 1917 Whyte, a medical missionary in China, called atten-

tion to the occurrence of thromboangiitis obliterans in natives of that country (41) and in the same issue of the Chinese Medical Journal an editorial urged surgeons in the Orient to attempt elucidation of the problems connected with the condition. In 1920 Ludlow (42), missionary surgeon at Severance Hospital in Seoul, reported that before 1917 he had seen five typical cases of the Buerger syndrome and described in detail four other patients seen in the three years since 1917. In none was there a history of frostbite, trauma or use of carbolic acid dressings. Diabetes and leprosy were excluded. The vegetarian diet of the Korean was noted. Ludlow contributed information on forty cases which were referred to in the monograph of Brown, Allen and Mahorner (43) in 1929. Ludlow expressed the belief that "the prevalence of the disease throughout Korea is much greater than the number of cases recorded would indicate." A series of fifty personally observed cases was reviewed by Ludlow in 1931 (44).

In 1925 Meleney and Miller (45) reported on twenty-five cases in Chinese in Peking and stated that "thromboangiitis obliterans is found extensively in China among the Chinese."

Snapper, in his *Chinese Lessons to Western Medicine* (14), commented on the exceeding rarity of senile and diabetic gangrene, and coronary sclerosis with angina pectoris and myocardial infarction. Furthermore he stated that 117 cases of thromboangiitis obliterans had been observed at Peiping Union Medical College Hospital between 1921 and 1940—113 in Chinese and four in Russian patients. Males accounted for 114 of 117. He concluded that thromboangiitis obliterans was the most frequent cause of gangrene of the lower extremities. He thought phlebitis was probably less frequent in Chinese patients with thromgoangiitis obliterans than in the West.

In 1931 Noble (46) reported cases in Siam. The present frequency of the Buerger syndrome in India would appear to be relatively high as indicated by personal communications (47) and by the fact that Singh and Brara were able to assemble thirty-six cases for a particular drug trial reported in 1960 (48). Sen reports (47) that the Buerger syndrome is "quite frequent" in surgical practice in Bombay.

In Singapore between 1953 and 1961, Yong (49) observed thirty-nine cases of obliterative arterial disease with onset of symptoms between ages 22 and 43 years. All but one were male. Twenty-three were Chinese (including the one female), eleven were Indians, three Malays, one Scotsman and one Eurasian. A majority had involvement of all four extremities.

"Blackfoot disease" of Taiwan is clearly distinct from the Buerger syndrome.[3] There is no age or sex preference; the disease is confined to the

[3] Through the courtesy and with the assistance of Dr. R. Quentin Blackwell and Capt. Francis M. Morgan, NAMRU #2, and of Prof. T. C. Kao, National Taiwan University,

most distal parts of the extremities; it occurs in the population of a restricted rural area of Formosa; calcification of distal arteries is a frequent feature.

Atheromatous disease in the aorta and iliacs has been suggested as a mechanism for occlusion distally in the extremities producing the Buerger syndrome. Presumably one of two possible mechanisms operates: 1) Embolism of atheromatous material from ulcerated aortic lesions, and 2) thrombosis distally due to interference with flow at the bifurcation of the aorta or in the iliacs.

As stated above, in two patients with disease limited to the legs and with a history of trauma to the sacral area the site of primary difficulty was in the iliac vessels as established by aortography. It is likely that the process in the other patients is different. Although aortograms were performed in only a few patients, mainly those in whom signs referable to the femoral arteries were discovered, it is difficult to imagine that "silent" disease of the aorta and iliacs was present in most of the cases for the following reasons:

1. In the population from which the cases were drawn aortic atherosclerosis at necropsy is at the most only mild (see above).

2. Arm involvement, present in a majority, would be even more difficult to account for on the basis of aortic involvement.

3. Femoral arteriograms, although an imperfect substitute for iliac demonstration by aortography, revealed no abnormality at this level.

The heart as a source of emboli to the extremities can probably be excluded by the absence of cardiac symptoms and by normal chest roentgenogram and electrocardiogram.

The histology of Buerger's disease has classically been described in three stages, acute arteritis, organizing thrombus and recanalization. The failure to find clear indications of acute arteritis in amputation specimens (and sometimes the findings of atherosclerosis) has been a serious handicap to acceptance of Buerger's disease as an entity. Good evidence on the stage of acute arteritis in the Buerger syndrome is provided by Ishikawa and Kawase (50) who have in studying a large number of such patients seen five at a stage when a radial artery was swollen and tender with reduced or absent pulse. Biopsy of the artery at this stage revealed an arteritis with "round cells" involving all layers of the vessel wall.

Racial susceptibility: In the cases of frostbite during the Korean War one of the most striking findings—in fact almost the only definite epidemiologic feature—was the greater susceptibility of the Negro (17). The dif-

one of us (W.S.H.) examined twenty-six patients with "blackfoot disease" in Taiwan and reviewed histopathologic, epidemiologic and experimental studies of the disease being conducted by the persons mentioned.

ference had no evident basis except race. We obviously had no way to evaluate whether the frequency of the Buerger syndrome was higher in Koreans than would have been the case in Caucasians living under identical circumstances.

The findings of much higher titers for cold hemagglutinins in Mongolian subjects than in either whites or Negroes may be pertinent (17).

Ergot: Ergotism is one of the classic etiologic theories in the Buerger syndrome (51). Several observations suggest the possible value of further study of the role of *Claviceps purpurea* or other fungi containing vasoactive substances: The association of the Buerger syndrome with low socioeconomic class; the fact that the higher the socioeconomic class the higher is the grade of polish on the rice eaten by Koreans; the fact that barley (which is harvested from the rice paddies in the late spring before the rice seedlings are set out) is eaten as a partial substitute for rice by the lowest socioeconomic classes; the fact that six of sixty-two cases in the larger series resided in the islands off the southern coast of Korea where barley is eaten in larger amounts.

Ergotism (51) possibly differs in important respects from the Buerger syndrome: cerebral symptoms are conspicuous; the patients describe sensations like insects creeping under and over the skin; blood pressure is likely to be elevated; older persons are more frequently affected than the young; females are as vulnerable as males.

Loss of pulse as a symptom: At least three patients volunteered the information that they themselves noted loss of pulse before seeking medical attention. Oriental medicine places great emphasis on the pulses (52), and in the Orient even the layman is relatively sophisticated about the pulse. It is said that when an Oriental person feels ill he feels his radial pulse much as in the West a layman might take his temperature. It is likely that the early and frequent recognition of "pulseless disease" (Takayasu's disease) in the Orient is due in part to this preoccupation (53) although also the disease is clearly more frequent there (34). In a group of forty-eight patients with pulseless disease studied by Sano and Shimizu (54, 55) loss of radial pulse was a presenting complaint in about a third.

In an editorial entitled "Thromboangiitis obliterans: fact or fancy" Wessler (4) defended the view that "the disease originally described by Buerger is indistinguishable from atherosclerosis, systemic embolization, or peripheral arterial or venous thrombosis, singly or in combination." Inclusion of the last, "arterial and venous thrombosis," leaves no room for disagreement since thrombosis is, after all, at least part of the process leading to circulatory insufficiency in the Buerger syndrome whatever the cause. We feel that the experience in Korea and Japan effectively excludes atherosclerosis and systemic embolism as causes of most cases of the Buerger syn-

drome. It seems improper, however, merely to label these "arterial thrombosis." It is more desirable to try to determine the several etiologic and pathologic factors in thrombosis leading to the Buerger syndrome. In any group of cases and even in individual cases these factors (loosely referred to as "the cause") may be multiple. In the Buerger syndrome seen in various parts of the world exposure, trauma, or tobacco may be factors disposing to intravascular thrombosis. The possibility of a progressive, fundamentally inflammatory process which is only initiated or aggravated by these factors must also be entertained and investigated.

In some of the editorial comments (5, 7) on the papers of Wessler *et al.* (3), Gore and Burrows (2), and Fisher (1), it may have been inappropriately suggested that Buerger's disease is not an entity. The real issue is whether primary angiitis underlies cases of the Buerger syndrome. The observations in the Orient provide neither support not refutation for the angiitis hypothesis. Unquestionably, however, there exist in the Orient and in the United States (56) occasional cases of the Buerger syndrome, representing an entity distinct from other categories of vascular disease and legitimately termed Buerger's disease. Its etiology and pathogenesis deserve study.

SUMMARY AND CONCLUSIONS

1. The Buerger syndrome—occlusive peripheral vascular disease with the clinical picture described by Buerger—occurs in relatively high frequency in Japan and Korea and probably in other parts of the Orient. It affects almost exclusively males. Onset is before 35 years of age in a majority of cases. The patients are almost without exception smokers, usually heavy smokers. The upper extremities as well as the lower extremities are affected in a significant proportion of cases. Thrombophlebitis occurs in some cases.

Auscultation was found useful in identifying involvement of the iliac and femoral arteries. A frequent arteriographic finding in cases with involvement of the arms was persistence and dilatation of the interosseous artery with occlusion of the radial and/or ulnar arteries. Occasionally a pulse in a perforating branch of the interosseous artery was palpable at the wrist and communications from the interosseous artery to the ulnar artery were identified arteriographically in some. In the legs a comparable finding —a perforating branch of the peroneal artery communicating with the terminal portion of the anterior tibial artery—was observed. The deep femoral artery was occluded in two patients.

2. In Korea and Japan the Buerger syndrome occurs predominantly in farmers and laborers, persons of lowest socioeconomic status.

3. Other impressive epidemiologic features are exposure and trauma, and a low level of dietary fat and protein consumption.

4. In a series of twenty-eight cases two patients had occlusive disease in

the iliac arteries with symptoms starting at the age of 33 and 35 years and probably secondary to trauma to the low back and sacrum. These two cases appear to represent a condition distinct from the others.

5. In a majority of the cases atherosclerosis, embolism and occlusive disease in the aorta and iliacs cannot account for the process. Most of the cases studied appear to represent a distinct category of vascular disease which can legitimately be termed *Buerger's disease*.

6. Histopathologic study of amputated specimens from nine Korean males revealed nonspecific organizing and recanalizing occlusion in eight. In one changes consistent with subacute angiitis were observed.

7. Multiple factors in the series of cases and perhaps even in individual cases may be responsible for the initiation and perpetuation of a process which is, within limits, progressive.

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On returning to the United States in the Summer of 1960, Dr. Paul S. Crane, Director of the Presbyterian Medical Center, Chonju, Korea, was surprised to discover that "Buerger's disease," which he had treated in many native Koreans, was no longer considered to be a distinct entity. Dr. Richard T. Shackelford, Baltimore, called Crane's observations to our attention. By correspondence and review of published observations the impression of a high frequency in the Orient was confirmed.

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APPENDIX I

1. Chung Too Sung (H3735), born in 1924, a farmer, had trouble beginning in the right leg while fleeing as a refugee in the Korean War (1951). For three months he lived under severe circumstances and during the latter half of this period his symptoms began. There was no trauma and no obvious frostbite. The other three extremities were progressively involved. The patient had smoked one to two cigarettes per day for one year before onset. Left lumbar sympathectomy was performed in 1958 and soon thereafter left "below knee" amputation. The patient was unable to work.

Examination in 1961 revealed absent femoral pulses bilaterally and bilateral occlusion of the ulnar arteries by palpation and by Allen test. The right foot was cold and red.

2. Suh Kee Soo (J8260), a farm laborer born in 1939, had no history of trauma or unusual exposure. The first symptom, a painful, red, hot nodule on the medial aspect of the right lower leg, was noted in December 1959 when the patient was 20 years old. Soon thereafter coldness and redness of the right fourth toe appeared and ulceration developed. There was right claudication. The other extremities had been asymptomatic except for occasional tingling in both hands and coldness in both legs when he sat on a cold stone.

No abnormal signs were found in the arms. Both popliteal pulses were present. The left dorsalis pedis pulse was full; however, the left fourth toe was amputated. No foot pulses were detectable on the right and the distal half of the right foot was red with early gangrene of the right great toe.

3. Oh Yu Lok (J7818), a farmer born in 1925, had a burn of the instep of the left foot at the age of 19 years and an abscess of the left calf area at the age of 16 years. Symptoms of claudication began in the left leg in 1958 and in the right leg in 1959. Numbness in the fingers of right hand had been noted in 1961. The left great toe was amputated and left lumbar sympathectomy done in January 1961. For three years he had been unable to work.

Examination in 1961 revealed absent pulses in the popliteal and foot vessels bilaterally. There were no objective signs of arm involvement.

4. Kim Nam Sung (I7414), born in 1924 and formerly a farmer, suffered severely as a prisoner of war in the Korean War. Escaping he walked many miles from North Korea carrying a younger sister on his back. He was in the Korean army from 1954 to 1956 and about 1956 may have sustained frostbite of the left foot while on guard duty at the front line. He smoked heavily. Symptoms of the Buerger syndrome began early in 1959 with pain and swelling in the left third and fourth fingers. Three months later the same symp-

toms appeared in the left great toe and to some extent in the toes on the right. The right arm had escaped symptoms. Left lumbar sympathectomy was performed in November, 1959, followed two weeks later by left "above knee" amputation. Cervical sympathectomy, performed in September, 1960, gave benefit. He did sheet metal work in a rehabilitation center.

Examination in 1961 revealed changes in all four extremities: Occluded right ulnar, occluded left radial with reduced flow in the left ulnar, absent right popliteal and posterior tibial pulses. The nail of the left fifth finger was better preserved than any of the others. The right foot was cold below the ankle.

5. Pae Kil Sun (K715), born in 1931, formerly a machine operator in a rice mill, began in 1954 with claudication and pain in the toes on the left. Five months later similar symptoms began in the right leg, and symptoms in both arms began in 1955. The tips of

FIG. 5A.

FIG. 5. Patient 5. See text. A and B. Right femoral arteriogram. See text. C. Left brachial arteriogram. See text.

the first three toes on the left, of the first four fingers on the right and the first three on the left were spontaneously amputated. Right lumbar sympathectomy was performed early in 1961. He had been unable to work for seven years.

Examination in 1961 revealed no popliteal or foot pulses on the right and no foot pulses on the left. Both ulnar pulses and the left radial pulse were not palpable. The right radial pulse was weak.

The right femoral arteriogram (Fig. 5A) shows a gradual narrowing of the femoral artery at the point indicated by the arrow. Lower in leg (Fig. 5B) there is slight narrowing in the popliteal area (see arrow). All the arteries in the upper or mid portion of the lower leg have become occluded and the circulation has been imperfectly taken over by collateral vessels which show marked corkscrew tortuosity as they approach the ankle.

FIG. 5B.

FIG. 5C.

The left brachial arteriogram (Fig. 5C) shows occlusion of both the radial and the ulnar arteries with blood supply to the hand exclusively via the interosseous artery. Filling of an attenuated palmar arcade via a tortuous branch of the interosseous artery (see arrow) can be seen.

6. Pak Tong Man (J7762), farmer born in 1934, was in the Korean army from 1957 to 1959. By that time he had already noted, beginning in 1956, claudication of the right leg followed shortly after by symptoms in the left leg. Numbness and coldness were present in both feet. About 1958 the patient noted disappearance of his right radial pulse. The patient was a heavy smoker. He had not worked for three years.

On examination in 1961 the right radial artery was found to be occluded by palpation and by Allen test. There were no definite trophic changes in fingers, however. Over the right femoral artery there was a loud systolic bruit and a conspicuous thrill. No popliteal or foot pulses were palpable in either leg. However, both feet were warm. (Bilateral lumbar sympathectomy had been performed in 1960.) The right great toe had been amputated.

FIG. 6. Patient 6. Right brachial arteriogram. See text.

The right brachial arteriogram (Fig. 6) reveals obstruction of the radial artery near its origin. The ulnar artery shows a tapering occlusion which was present in several successive views. Since the ulnar pulse was thought to be palpable, the apparent occlusion of the ulnar artery may be a matter of spasm. Alternatively, it is possible that the pulse in a branch of the interosseous artery was felt.

7. No Sung Pum (J1035), a farmer born in 1937, escaped military service because of "Buerger's disease" which began in 1951. He was a refugee in the Korean War but did not suffer severely. He smoked rather heavily from the age of 15 years. He started smoking about the same time his circulatory difficulty was beginning. Symptoms referable to all four extremities have occurred, beginning with the right leg and followed by the left leg two years later and by both arms five years after first onset in 1951. Bilateral lumbar sympathectomy and "above knee" amputation on the left have been performed.

Examination in 1961 revealed occlusion of the right radial and left ulnar arteries as indicated by palpation of pulses and by the Allen test. The right foot pulses were not palpable.

A right brachial arteriogram (Fig. 7) shows occlusion of both the radial and the ulnar arteries near their origin. The radial pulse was absent and occlusion of the ulnar artery is unquestionable since there are collaterals coursing down the ulnar side of the forearm (arrow) and one (arrow) crossing the distal end of the ulnar bone from the interosseous artery. What was interpreted as ulnar pulse may have been a pulse in the last mentioned collateral or some other part of the interosseous distribution.

8. Pak Jae Sung (G7377), born in 1917, a farmer without history of unusual exposure,

FIG. 7. Patient 7. Right brachial arteriogram. See text.

frostbite, or military and refugee history, began in 1956 with left calf claudication relieved by rest. Symptoms (coldness, tingling, pain) then followed in both hands, worse on the left. Weakness in the lef leg and gangrene of the left great toe necessitating amputation developed in 1957. Left lumbar sympathectomy was performed two months after the amputation. The patient had smoked heavily since about 1937. He was unable to work and was supported by his son.

Examination in 1961 revealed occluded left radial and right ulnar arteries (by palpation and Allen test), aberrant dorsal ulnar artery on the right, absent foot pulses on the right, and absent popliteal and foot pulses on the left. The legs were spindly in the lower portion.

The garb shown in Fig. 8A is that typical of the Korean farmer. The shoes are the rubber ones characteristically worn.

The right femoral arteriogram (Fig. 8B) shows obstruction of all branches of the femoral artery in the upper and mid-thigh. The common femoral is intact. (Occlusion of the deep femoral artery is uncommon.) Typically tortuous collaterals are seen. Two views of the right knee area and lower leg (not shown here) showed filling of the popliteal artery (apparently through collaterals) but there was further occlusion in the thigh area with formation of collateral vessels in the mid-lower leg.

FIG. 8A.

FIG. 8. Patient 8. A. Overall appearance. Note rubber shoes. B. Left femoral arteriogram. See text.

9. Im Wol Sung, a farmer born in 1933, was in the Korean army from 1954 to 1956 but had no unusual exposure, frostbite or trauma. Symptoms began in the right leg in 1957, in the left leg in 1958 and in both arms in 1959. Amputation of the right great toe and bilateral lumbar sympathectomy were performed in 1958, apparently with considerable improvement in leg symptoms, permitting him to return to work. He smoked heavily from the age of 15 years. For the brief periods when he stopped smoking he noted relief of his arm symptoms.

On examination in 1961 the radial and ulnar pulses were absent in both arms. On Allen tests all flows to the hand were deficient; on the right the radial flow was poorer than the ulnar and on the left ulnar flow was poorer. The hands were very cold and

FIG. 8B.

sweaty. Incipient gangrene was present in the fingers of both hands. The feet were warm, probably as a result of the sympathectomy, but showed no pulses.

The right femoral arteriogram (Fig. 9) shows extensive occlusive disease in the lower leg (e.g., upper arrow). There are many fine collateral vessels which are tortuous with corkscrew configuration near the ankle (see lower arrow).

10. Yu Jung In, born in 1916, a seaman who also worked as a fisherman, had the onset of claudication in the right leg, with coldness, numbness, and pain in the toes of that foot, in 1955. In 1956 symptoms began in the fingers of the right hand. Pain was very severe, disturbing sleep. Spontaneous amputation of the tips of the second, third and fourth fingers of the right hand occurred. Cervical sympathectomy was performed on the right in 1959, with great symptomatic relief. The left hand remained asymptomatic but severe symptoms in the left leg with gangrene of the great toe developed in 1957. He had

FIG. 9. Patient 9. Right femoral arteriogram. See text.

smoked heavily from the age of 20 years. He was unable to work from 1957 to 1959 because of his "Buerger's disease" but since that time has been able to work again.

Examination in 1961 revealed absent foot pulses on the right and absent popliteal and foot pulses on the left. Mild varicosities were found on the posterolateral aspect of the left lower leg. In the right arm the radial pulse was weak and the ulnar pulse was not felt. However, on Allen test better filling occurred through the ulnar than through the radial. The left ulnar pulse was absent, the flow through the left ulnar artery was by Allen test demonstrably poor and the left fifth finger was much cooler than the rest of the hand.

11. Pak Jong See (I4466), a farm laborer born in 1915, worked under severe circum-

FIG. 10A.

FIG. 10. Patient 11. A and B. Right brachial arteriograms. See text. C. Left femoral arteriogram. See text.

stances as a laborer in the Korean War for one year before the onset of symptoms in the left leg in 1952. Symptoms in the right leg began in 1953 and in the right arm in 1958. The left arm has remained asymptomatic. No surgery had been performed. However, spontaneous amputation of the toes of both feet had occurred. In fact, in the right foot a spontaneous transmetatarsal amputation had occurred with a wound persisting since 1954. The patient had smoked heavily from the age of 18 years.

On examination in 1961 the right radial pulse was found to be absent with impaired radial flow on Allen test. No popliteal or foot pulses were present in either leg. A loud systolic bruit and thrill were discovered over the right femoral artery.

The right brachial arteriograms, of which three are reproduced here, show a normal brachial artery down to the point of its antecubital bifurcation (Fig. 10). Here the radial artery is occluded but the interosseous and ulnar persist. The ulnar is abnormally tortuous at the wrist (Fig. 10B) and not as large as one would expect in view of the fact that it is the only blood supply to the hand. Although the left common femoral artery (Fig. 10C) is not notably abnormal, all its branches are occluded near their origin and many relatively fine, tortuous collateral vessels are seen in the thigh. As stated above (case 8), occlusion of the deep femoral artery is unusual in the experience of most arteriographers.

12. Yue Tae Kyo (G7941), a farmer born in 1915, worked as a forced laborer for the Japanese in Kyushu from 1942 to 1945. Symptoms began in 1956, one year after his return to Korea, with pain and ulceration of the right great toe which, he felt, was related to trauma. The ulceration persisted two months before the toe was amputated. In 1951 symptoms began in both hands. Claudication began in the left leg in 1958. In 1957 right

FIG. 10B.

lumbar and left cervical sympathectomies and right "below knee" and left third finger amputations were performed. Other fingers were amputated by irregular doctors.

On examination in 1961 radial and ulnar pulses were absent on the right with poor filling on Allen test. On the left an axillary arterial pulse could be felt but no brachial or antecubital pulse. A weak radial pulse could be felt. The Allen test could not be performed on the left because of the multiple finger amputations. Amputations had been necessitated by involvement of both hands and the right leg. The patient also had claudication in the left leg and absent popliteal and foot pulses.

13. Min Byong Koo (14790), a farmer born in 1914, had not worked for many years because of his "Buerger's disease." In 1942 there was an episode of possible frostbite of both feet. In 1944 severe pain in the right fifth toe began and one year later pain in the left second toe. Both legs were amputated below the knee in 1947. Multiple episodes of gangrene in the fingers necessitating many amputations occurred despite bilateral cervi-

FIG. 10C.

cal sympathectomies. From the age of 20 years he has been a heavy smoker. He devised ingenious boots, shod with part of the casing of tires, which permit him to walk on his knees (Fig. 11B).

Examination revealed marked mutilation of all four extremities, as shown in the photographs (Fig. 11A). Femoral pulses were normal but no popliteal pulses were felt.

FIG. 11A.

FIG. 11B.

FIG. 11. Patient 13. A. Four extremity involvement is demonstrated. B. Shown here is the shoe-like device by means of which the patient walked on his "below knee" amputation stumps.

The left radial pulse was very weak and no ulnar was palpable. The right radial pulse was also weak and the ulnar was missing but an accessory dorsal ulnar artery had a pulse. The Allen test could not be performed for reasons obvious from the photographs.

The four extremity involvement is graphically illustrated in the figures (Fig. 11). Also shown is the type of shoe with which the patient could walk on his "below-knee" stumps.

14. Han Moon Kyo (G990), a farmer who was born in 1924 and who was still able to work, fled from near Chonju when the invasion from North Korea occurred but did not suffer particularly. In October, 1956, while on a train trip he sustained a frostbite followed by pain, coldness, and discoloration in the left great toe which was amputated in a local clinic. Left lumbar sympathectomy was performed in 1957. No symptoms occurred at any time in the other three extremities. He had smoked heavily from the age of 21 years.

On examination in 1961 he was found to have, despite lack of symptoms, absent popliteal and foot pulses on the right and apparently occluded right and left ulnar vessels. The right foot was cold.

A right femoral arteriogram (Fig. 12) shows obstruction just below the knee and tortuous collateral vessels. At the level of the knee joint an indentation in the popliteal artery (arrow) suggests atheroma.

15. Pak Un Kyne (K118), born in 1928, a farmer, was in the Korean Army 1951 to 1954. He sustained no frostbite but in 1953 had trauma to the right index finger, which was amputated, and pieces of shrapnel lodged in the left forearm (Fig. 13). Immediately after the last injury the patient noted loss of the left radial pulse. Coldness and muscle atrophy in the hand and fingers on the left were first noted about 1956. Pain in the left lower calf began early in 1958 with pain and discoloration of the left great toe appearing one year later, accompanied by alternating hot and cold sensations in the third and fourth toes of the right foot. The left great toe was amputated and left lumbar sympathectmy was performed in January 1961. The patient smoked heavily from about 1951.

Examination in 1961 revealed occluded left radial and right ulnar arteries (by palpation and by Allen test) and no pulses in and below the popliteal arteries bilaterally.

FIG. 12. Patient 14. Right femoral arteriogram. See text.

Figure 13 shows a) the location of the shrapnel in the left arm, b) a drawing of the arterial tree as derived from an arteriogram not shown here because it could not be clearly reproduced. The radial artery stops short at the site of one bit of shrapnel. The interosseous artery is dilated, as in many of the other cases, and probably is responsible for an important part of the supply to the hand.

16. Chung Nam Hyun (K136), farmer born in 1921, had no history of unusual exposure or frostbite. While in jail in 1945, he was struck heavily in the sacral area (see p. 253). In 1956 at the age of 35 years symptoms began in the left leg. However, the right leg, in which symptoms were later in onset, gave the patient more trouble. There were never any symptoms relating to the upper extremities. Bilateral lumbar sympathectomy had been performed. He had smoked more than twenty cigarettes a day from the age of 20 years.

On examination in 1961 neither ulnar pulse was felt and the left radial pulse was less full than the right. However, Allen test suggested no abnormality. The right femoral pulse was present but weak. Over the right femoral artery a prominent systolic bruit and thrill was discovered. No popliteal or foot pulses were palpable in either leg. The left second toe had been amputated. Both feet were warm.

The aortogram (Fig. 14) shows that the right external iliac artery is markedly narrowed and post-stenotic dilatation of the femoral artery is demonstrated. There is a long narrow-

FIG. 13A.

FIG. 13. Patient 15. A. location of shrapnel in left forearm. B. Tracing of left brachial arteriogram. See text.

FIG. 13B.

ing of the left femoral artery with a post-stenotic dilatation distal to the narrowed zone. The main arteries in both legs above and below the knees appeared normal in views not shown here. There were, however, tortuous vessels (collaterals) in the left thigh.

17. Yune Chang Soo (J5796), a farmer born in 1918, was struck in the sacral area and about the knees by a communist, about 1946. Symptoms in the form of an "electric sensation" in the left thigh followed by cyanosis and redness in the toes began in 1952. Eighteen months later the same symptoms began in the right leg. There were no symptoms referable to the arms, except for occasional coldness in both hands. Bilateral lumbar sympathectomy was performed in August 1960. Right "above knee" and left "below knee" amputations were performed in August 1960 and January 1961, respectively. The patient had been a heavy smoker from the age of 16 years.

Examination in 1961 revealed no pulses in the femoral arteries of either leg. No left popliteal pulse could be felt. In the arms both radial and ulnar pulses were full; the hands were warm with full veins; the Allen test showed good filling through both ulnar and radial arteries.

The patient's mode of locomotion is shown in Figure 15A. The aortogram (Fig. 15B)

FIG. 14. Patient 16. Translumbar aortogram. See text.

shows complete obstruction of the left iliac and marked narrowing of the right iliac near its origin.

18. Kim Chun Ho (K1126), a government clerk born in 1918, had first symptoms, pain and numbness in the fourth finger of the right hand, about 1946. One year later symptoms began in the left hand and two years later in the right leg. Finally symptoms began in the left leg four years after first onset. He had been a heavy smoker from the age of 13 years. Multiple sympathectomy and amputation procedures have been performed beginning in 1951. The patient was still employed in 1961.

On examination in 1961 the right radial pulse was weak with poor flow on Allen test. The left radial pulse and radial flow by Allen test were totally lacking. The first and fifth toes of the right foot and the fifth toe of the left had been amputated. The right third and fourth fingers were gangrenous at the tips. In the left leg popliteal and foot pulses were absent and the right foot pulses were also absent.

19. Ha Pong Ho (G2620), a former construction company clerk and foreman, had onset

FIG. 15A.

FIG. 15. Patient 17. A. The patient, who has amputation of both legs, is carried by a relative. B. Translumbar aortogram. See text.

in 1952 of a drawing sensation in the left calf with exertion. He had sustained a bullet wound of the left calf in 1950. Beginning in 1960 he noted fatigue of the right hand in writing. For two months early in 1961 he had noted claudication in the right leg. The patient had smoked heavily from the age of 18 years. He had been unable to work for three years.

On examination in 1961 there could not be said to be any definite objective evidence of deficient blood supply to the hands. The right radial artery had reduced flow by Allen test but an accessory dorsal radial branch had good flow. Neither ulnar pulse was palpable, but on Allen test ulnar flow seemed good. Both feet were cool, the right more than the left. Coldness extended to the mid-shin area on the right and to a point above the knee on the left The left great toe had been amputated. On the posteromedial aspect

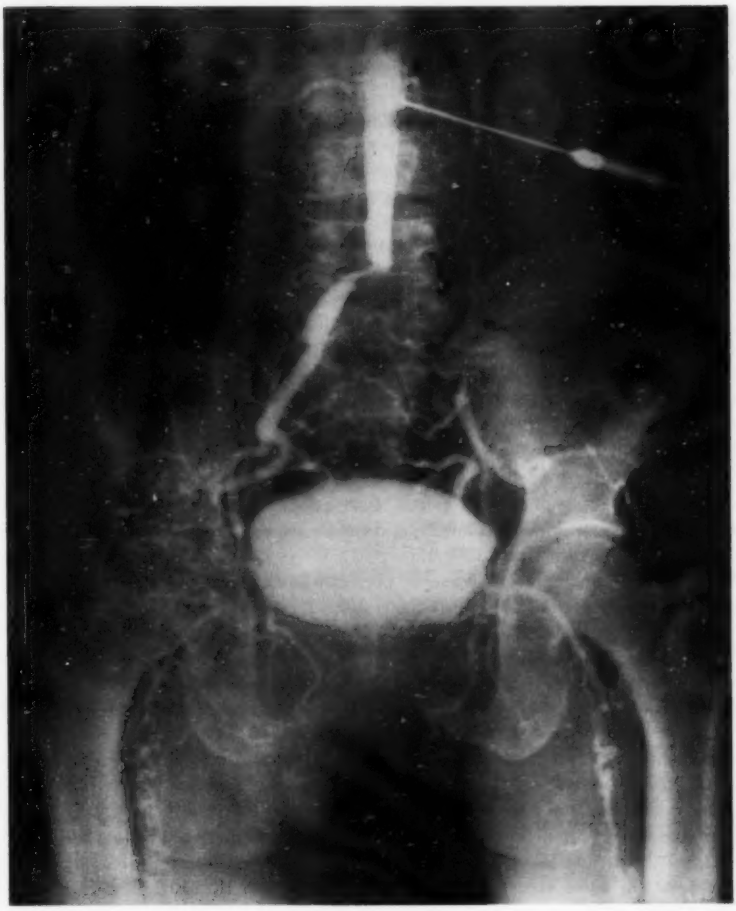

FIG. 15B.

of the right calf an ill-defined nodule about 1 centimeter in diameter was attached to the fascia of the underlying muscle. It was excised for biopsy. Histologically the nodule proved to be of chronic inflammatory nature. Although vessels were identified and were involved in the inflammatory change a primary angiitis could not be determined with certainty.

20. Cho Byong Kae (J6020), farmer born in 1928, recalls frostbite of both feet during the Korean War (about 1951). In 1954 claudication began in the right calf and thigh. In 1958 similar symptoms began in the left leg and gangrene of the left toe developed. He applied lye to the toe and removed the gangrenous member himself. Late in 1960 tingling sensations of the left hand were noted. Bilateral lumbar sympathectomy was performed in 1960. The patient had smoked from the age of 23 years.

Examination in 1961 revealed no popliteal or foot pulse on either side. The left

radial and right ulnar pulses were lacking. A chronically ulcerated area occupied the site of the first and second toes and extended onto the dorsum of the foot. There was pronounced atrophy of the left leg musculature and a moderate flexion contracture of the left knee. There were mild varicosities bilaterally.

21. Cho Byong Too (J6691), farmer born in 1914, had no clear history of unusual exposure, frostbite, or injury of the extremities. He had smoked heavily from the age of 20 years. As will be noted, symptoms of Buerger's disease began before the onset of smoking. Claudication in the left leg and coldness and pain in the toes of the left foot began at about the age of 16 years. Seven years later similar symptoms began in the right leg and three or four years after that numbness and coldness in the fingers of both hands with loss of several fingernails. In 1961 the left leg was amputated below the knee because left lumbar sympathectomy performed in 1960 had failed.

Examination in 1961 revealed absent popliteal, posterior tibial and dorsalis pedis pulses. The right radial pulse was weak and both ulnar pulses were absent with poor ulnar filling on Allen test. The tip of the right great toe had been spontaneously amputated. All the fingers showed marked atrophy of the distal pads with resulting tapering of the fingertips.

22. Ko Un Joo (K262), farmer born in 1927, has had symptoms and signs only in the left leg, beginning early in 1960 with pain in the middle toe, followed by color change in the left foot and claudication. Left lumbar sympathectomy in 1960 resulted in great improvement although mild claudication persisted. He was a light to moderate smoker beginning at the age of 20 years.

Examination revealed all pulses to be perfectly intact including those in the left foot.

23. Yue Chul Kyne (H3143), born in 1907 and a farmer who still worked, sustained a sprain of the left foot from a fall at the age of 10 years. Symptoms in all four extremities have occurred beginning in 1950 with coldness and color changes in the middle finger of the right hand. In 1951 claudication was first noted in the left legs. In 1956 he developed coldness in the right foot. Bilateral lumbar sympathectomy was performed in 1958 and right "below knee" amputation thereafter. The patient felt considerable improvement in the right leg with sympathectomy and his disease appeared to be arrested. He was a heavy smoker from about 1928 until 1958 when he stopped completely.

Examination revealed a cold left thigh and stump although the femoral pulse was full without bruit. On the right leg all pulses except the posterior tibial were present. In both arms the ulnar pulses were absent and bilaterally no ulnar filling was observed on Allen test.

24. Hur Mo (J3867), a farmer born in 1927, began with symptoms in both legs in 1956, followed by symptoms in the right hand about one year later. Left lumbar sympathectomy was performed in 1960. The patient was a heavy smoker from the age of 23 years. There was no history of unusual exposure and none of trauma.

On examination in 1961 the right antecubital pulse was found to be very weak and no blood pressure could be determined in the right arm by the usual method. The right radial pulse was very weak with poor filling on Allen test. The right ulnar artery appeared to have no flow. Although the left arm was asymptomatic the radial pulse was absent. Both hands were cool. No pulses were palpable in the popliteal and foot arteries of both lower extremities. The left foot was warm presumably because of the left lumbar sympathectomy. The right foot was cool up to the mid-shin. Spontaneous amputation of the tips of the toes on the right had occurred.

Left brachial arteriograms showed obstruction of the radial artery.

25. Cho Ik Whan (J7285), farmer born in 1926, had no history of unusual exposure or trauma. About 1951 symptoms began in both hands, with coldness and numbness which were exaggerated by cold and worse on the left. About 1954 symptoms began in the left leg and about 1956 in the right leg. Four toes of the right foot were amputated surgically and in 1960 bilateral lumbar sympathectomy was done. He had smoked heavily from the age of 20 years. He was not able to work.

On examination in 1961 no pulses were found in the popliteal vessels and below in both legs. Both feet were cool with a gradient extending higher on the right lower leg than on the left. All the right toes had been spontaneously or surgically amputated. The toes on the left were abnormally red and shiny and the nails were thought to show trophic changes. Venous varicosities were present on the posterior aspect of the left lower leg. In the arms the right ulnar pulse was absent and the left radial pulse weak; the Allen test showed slow filling through the corresponding vessels.

26. Oh Keel Won (K1632), a farmer born in 1913, began with coldness and pain in both feet about 1957. Medical attention was not sought until March 1961 when symptoms of lockjaw had their onset. No signs of abnormal circulation in the arms were found. Both feet were gangrenous, the right showing more marked changes. Popliteal and foot pulses were absent bilaterally. Below knee amputation on the right and debridement of the ulcerations of the left foot were performed on the right and tetanus antitoxin administered. Histologically the vessels removed with the amputated part showed no changes interpretable as indicating primary angiitis. The patient was discharged much improved twenty-four days after admission.

27. Lee Ki Bum (G2420), a farmer born in 1908, first noted coldness and hypesthesia in the left leg in January, 1956. Coldness in the left index finger followed shortly after. There were no symptoms referable to the right arm and leg. He had suffered no frostbite or unusual exposure but about 1938 accidentally amputated part of the right fourth and fifth fingers with a rotary saw. He smoked heavily from the age of 25 years. Left lumbar sympathectomy was performed in 1957. The patient was still able to work.

Although the right arm was asymptomatic the ulnar pulse was absent and no ulnar flow could be demonstrated by Allen test. Pulses and other evidence of arterial flow were normal in the left arm and right leg. Foot pulses were lacking on the left.

28. Chegal Nam Sool (I6230), the operator of a lumber shop, born in 1927, began with weakness in the left leg and pain in the toes of the left foot about 1956. Two years later ulceration and early gangrene developed. Then paraesthesia appeared in the left leg. For about a year he had had numbness in his hands and trouble working with his arms over his head. He smoked heavily from the age of 25 years. The patient was in the army from 1952 to 1954 but at no time had suffered unusual exposure. He was more prosperous than any other member of this series, eating meat almost daily, fish four or five times a month and eggs about ten times a month. He was able to conduct his business satisfactorily.

Examination in 1961 revealed on Allen test reduced flow in both the ulnar and the radial arteries bilaterally. No pulses were palpable in the popliteal and foot arteries bilaterally. The right foot was slightly cooler than the left. The left fifth toe was amputated. There was a small patch of discoloration at the lateral corner of the nailbed of the left big toe, suggesting early gangrene. The signs were interpreted as indicative of four-extremity involvement with onset at 29 years in a man who had not had unusual exposure.

The following two patients (two of the sixty-two in the larger series) returned for follow-up shortly after the authors left Chonju. However, they were studied in the same

manner by the persons who had assisted in the study of the other patients. The abstracts are presented here even though the results have not been included in the analyses.

29. Kim Teck Hi (H3580), born in 1923, a farmer, had onset of coldness in the right foot and claudication on the right about 1952. Above knee amputation was performed on the right in 1956. Also in 1956 symptoms began in the left leg; these progressed to the point that below knee amputation on the left was performed in 1958. In 1958 symptoms appeared in the fingers of the right hand and in 1959 in those of the left hand. From age 19 he had smoked about nine cigarettes a day.

The femoral pulse was absent on the right. By Allen test both ulnar arteries showed deficient flow.

Fasting blood sugar was 178 mg. per cent and serum cholesterol 238 mg. per cent. Mild diabetes mellitus may be at least a contributing factor in the vascular disease of this patient.

30. Im Jong Soo (J5037), a farmer born in 1920, had onset of left claudication in 1940. The left foot was amputated spontaneously (with the assistance of very hot saline). A below knee revision of the amputation was performed in 1960. In 1950 there was onset of pain in fingers of the left hand and gangrene of the left third and fifth fingers was noted first in January 1960. These required subsequent amputation and cervical sympathectomy. In 1953 symptoms began in toes of the right foot. The patient began smoking cigarettes (about twenty a day) at about the same time that the symptoms of vascular disease began.

On examination in 1961 the right radial and left ulnar pulses were absent with corresponding changes in Allen's test. The left femoral pulse was weak and left popiteal absent. Only the foot pulses were absent in the right leg.

EKG, serum cholesterol (200 mg. per cent), and fasting blood sugar (98 mg. per cent) were normal in this patient.

APPENDIX II

Cholesterol Values in Koreans

Buerger Syndrome			Comparison Subjects		
Age	Cholesterol determined in Korea (mg. %)	Cholesterol determined in Baltimore (mg. %)	Age	Cholesterol determined in Korea (mg. %)	Cholesterol determined in Baltimore (mg. %)
54	195	180	59	201	154
53	198	148	52	216	193
48	164	120	50	241	240
47	209	205	47	196	186
46	203	165	46	177	—
47	193	200	46	216	160
46	171	145	45	237	—
45	203	180	40	191	154
44	189	154	39	234	246
43	187	180	36	205	180
43	228	—	33	219	193
40	206	200	33	206	180
40	190	180	32	203	212
37	206	186	31	173	135
37	162	135	31	166	—
37	179	123	30	150	120
36	209	212	30	207	175
35	205	165	30	184	180
35	182	170	29	196	154
34	231	205	29	188	170
34	192	165	28	204	165
33	218	193	28	266	240
30	231	212	28	171	—
30	190	154	27	194	165
28	217	165	27	165	165
27	218	212	27	218	212
24	172	130	26	168	160
22	163	120	25	244	205
Mean 38.4 yr.	196.7 mg.% S.D. = 20.3	170.4 mg.% S.D. = 29.3	35.1yr.	201.3 mg.% S.D. = 27.2	180.8 mg.% S.D. = 31.5

Correlation coefficient of two sets of serum cholesterol determinations (those done in Korea and those done in Baltimore); 0.84.

PANCREATIC FUNCTION AND THE VISCOSITY OF PANCREATIC JUICE BEFORE AND DURING CORTISONE ADMINISTRATION

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Recently it has been recognized that acute pancreatitis may occur in patients coincident with the administration of corticotropin (ACTH) or adrenal steroids (1, 2, 3, 4, 5). In addition many patients who receive steroid therapy develop definite lesions in the pancreas in the absence of clinically recognizable pancreatitis (5, 6). A pathological change observed frequently in both groups is ectasia of the pancreatic acini and their collecting ductules; often inspissated secretions are deposited within them. Similar changes have been produced in experimental animals (7, 8). Because of these findings it has been postulated that steroid induced pancreatitis may result from obstruction of the acinar spaces or terminal portions of the collecting ductules due to an increased viscosity of the pancreatic secretions (6, 7).

The purpose of this report is to present the results of studies of pancreatic function and the viscosity of pancreatic juice in dogs before and during administration of cortisone.

METHODS

Using the method of Thomas (9, 10), a 2.5 cm. diameter plastic and metal cannula was permanently inserted into the duodenum opposite the main pancreatic duct ampulla in two mongrel dogs weighing 20 to 25 kg. The cannula opened onto the abdominal wall and when not in use was closed by a plastic cap. Total pancreatic secretions were collected by inserting a small 1.5 mm. bulb-tipped metal cannula into the pancreatic duct under direct vision. All experiments were performed after a twelve to eighteen-hour fast.

Studies of pancreatic function were accomplished using two methods of stimulation of the pancreas 1) feeding a 12 ounce can of horse meat and 2) injecting rapidly 4 units per kg. of secretin[1] and 4 units per kg. of pancreozymin[2] into separate veins. Following control studies, cortisone acetate[3] was given intramuscularly for six weeks in a dose of 5 mg. per kg. per twenty-four hours and the pancreatic function tests were repeated.

Pancreatic juice was collected directly into volumetric tubes immersed in an ice bath. Following the onset of flow the first 5 cc. of pancreatic juice was collected separately. All subsequent specimens were collected in separate 8 cc. volumes. The time required for collection of each sample was recorded. The viscosity, amylase activity and protein, chloride and bicarbonate content of each sample of pancreatic juice was measured.

[1] Secretin kindly supplied by Eli Lilly Co., Indianapolis, Indiana.

[2] Pancreozymin obtained from Boots Pure Drug Co., Nottingham, England.

[3] Kindly supplied by Merck, Sharp and Dohme, Philadelphia, Penna.

Viscosity was measured by pipetting 5 cc. of pancreatic juice into a #100 modified Cannon-Fenske-Ostwald viscometer (11) immersed in a water bath at 38°C. After temperature equilibration, the efflux time of the pancreatic juice through the lower reservoir of the viscometer was measured 5 times with a stop watch. Reproducibility was good (relative error less than 1 per cent). De-ionized water had an efflux time of 44.4 ± 0.2 sec. and isotonic saline 45.1 ± .1 sec. (mean ± 1 S.D.). With this viscometer the efflux time is directly related to the kinematic viscosity in stokes. In these experiments the viscosity was expressed in seconds of efflux time.

Total protein was measured with Biuret reaction by comparing the resultant optical density of the pancreatic juice to that of known concentrations of human albumin. Chloride was determined by the method of Cotlove *et al.* (12), bicarbonate concentration by the method of Peters and Van Slyke, (13) and, amylase by the method of Myers, Frier and Rasinski (14).

Clinical and Pathological Observations

During cortisone administration dog A lost 12.5 per cent of body weight and dog B 22 per cent. Serial determinations of serum amylase, glucose, urea nitrogen, cholesterol, total lipids and proteins remained unchanged. Glycosuria and proteinuria did not develop.

Dog A died unexpectedly during the sixth week of cortisone administration. At autopsy the pancreas was normal. No cause of death was found. Microscopic examination of the tissues showed no change except acute pulmonary edema. Autolysis partially obscured microscopic detail of the pancreas, however, no antemortum changes were identified.

Dog B developed an extensive dermatitis after decreasing cortisone at the end of the sixth week period. The animal was sacrificed one week later. Gross and detailed microscopic examination of the pancreas showed no lesions.

RESULTS

Studies Following Meal Stimulation

In dog B meal stimulation was studied four times during the control period and three times during the third to sixth week of cortisone administration. When the animal was fasting the flow of pancreatic juice was approximately 0.1 cc. per min. Immediately after food was given the rate of flow increased. In control studies the rate of flow reached an average of 1 cc. per min. in the fourth or fifth collection period and then decreased to initial rates. During cortisone administration flow rates following meal stimulation were increased and averaged 2 to 2.5 cc. per min. in the eighth collection period[4] (Fig. 1A). The increased flow and other changes noted during the cortisone studies were not related to the duration of cortisone administration.

The viscosity of pancreatic juice was highest in the first 5 cc. samples

[4] Using the t test for small samples the differences in the flow rates during the control and cortisone periods had p levels of .01, .3, .4, .3, .2, .05, .001, .02 (collections 1 through 8).

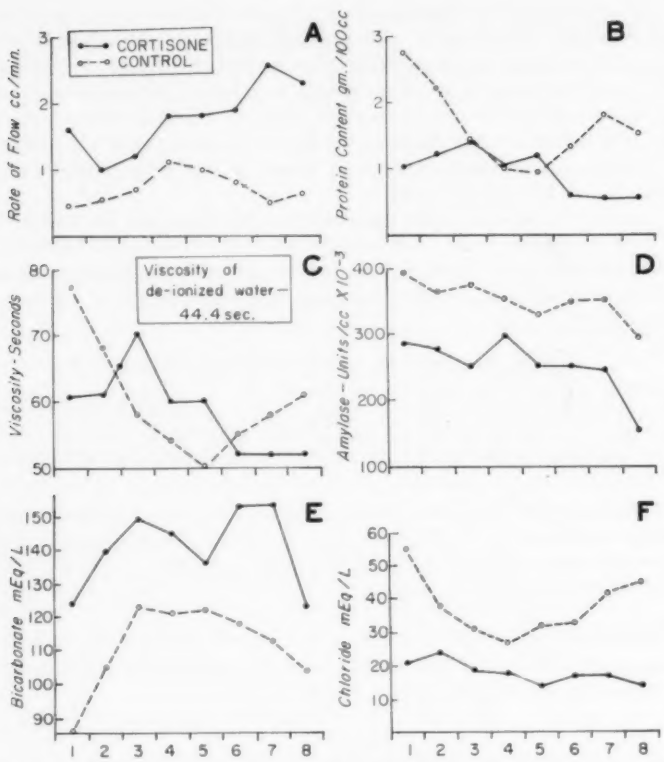

FIG. 1. Effect of cortisone on pancreatic juice following meal stimulation. The figures on the horizontal axis represent the individual collection periods.

(mean 77 sec.) and the successive 8 cc. samples followed the pattern seen in Figure 1C. During cortisone administration viscosity was more variable and was not significantly different from corresponding control collections. The first collection samples in the cortisone period were lower and showed the greatest difference from the corresponding controls (p level of 0.1).

The relationship between the rate of flow and the viscosity of the individual collections of pancreatic juice following meal stimulation is shown in Figure 2. During cortisone administration collections of pancreatic juice with viscosities similar to those of the control group were excreted with a distinctly increased rate of flow.

Cortisone also changed the relationship between the viscosity and protein content of the individual collections of pancreatic juice. Inspection of Figure 1B and Figure 1C suggested a direct relationship between viscosity and protein content. However, during cortisone administration collection sam-

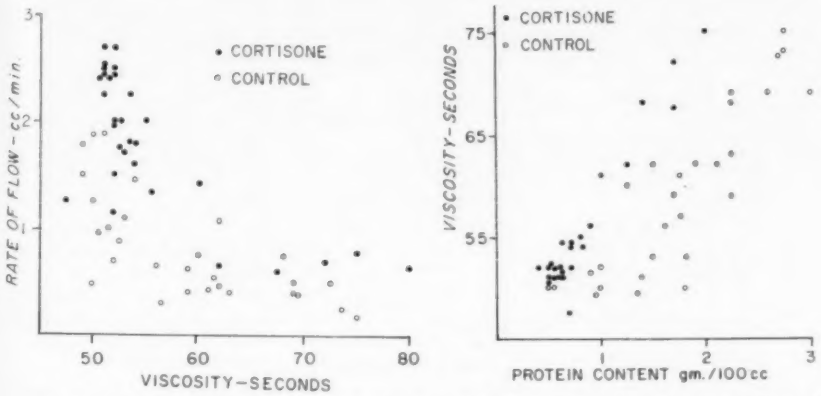

FIG. 2. Relationship between rate of flow and viscosity of pancreatic juice collected following meal stimulation. During cortisone administration collections of pancreatic juice with viscosities similar to control collections had distinctly increased rates of flow.

FIG. 3. Relationship between viscosity and protein content of pancreatic juice collected following meal stimulation. During cortisone administration collections of pancreatic juice with protein concentrations similar to control collections had greater viscosities.

ples with protein concentrations similar to control samples had consistently higher viscosities (Fig. 3).

The patterns of amylase activity, bicarbonate and chloride content are shown in Figure 1D–F. Amylase activity was consistently lower during the period of cortisone administration. Concomitant with the increased rate of flow during cortisone administration there was a higher bicarbonate and a lower chloride content in the pancreatic juice.

Studies Following Pancreozymin and Secretin Stimulation

In the fasting dog the rate and volume of pancreatic excretion was maximum following the rapid intravenous injection of 3 units of secretin per kilogram. The simultaneous injection of 4 units of pancreozymin per kilogram increased the viscosity of the secretions without further increasing the flow rate. Studies were performed using a standard dose of 4 units of secretin and 4 units of pancreozymin per kilogram. In the fourteen studies during the control period (ten in dog A and four in dog B) pancreozymin-secretin stimulation was given no oftener than once in twenty-four hours. During the cortisone period pancreozymin-secretin stimulation was first done four days after beginning cortisone and was repeated each week during the six weeks of cortisone administration. The changes produced by cortisone were not related to the duration of cortisone administration.

In the control studies the rate of flow of pancreatic juice following pan-

TABLE I

Measurements of Pancreatic Juice Following Simultaneous Pancreozymin and Secretin Stimulation During Control and Cortisone Periods

Collection		Flow Rate cc./min.		Viscosity Seconds		Protein gm./100 cc.		Amylase Units/cc. × 10^{-3}		Bicarbonate mEq./L.		Chloride mEq./L.	
No.	CC.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
						Dog A—Control							
1	5	2.1	0.3	82.4	17.5	2.5	0.7	314	59	109	23	39	9
2	8	2.0	0.6	54.3	1.9	0.8	0.4	204	97	127	12	22	7
3	8	1.5	0.6	49.6	2.8	0.3	0.5	105	54	127	11	21	7
4	8	1.0	0.6	48.2	1.3	0.1		83	61	120	19	25	16
						Dog A—Cortisone							
1	5	2.6	0.5	102.4	27.3	2.7	0.6	368	23	102	10	42	4
2	8	2.9	0.8	61.3	4.7	0.8	0.5	173	61	125	9	28	6
3	8	2.3	0.5	50.7	1.3	0.4	0.9	37	13	131	8	25	7
4	8	2.0	0.5	48.9	1.1	0.1		35	21	133	7	22	5
5	8	1.6	0.6	48.7	0.9	0.1		40	11	132	12	23	7
6	8	0.7	0.5	50.2	0.7	0.1		78	20	123	8	30	8
						Dog B—Control							
1	5	2.3	0.2	91.1	17.6	3.0	0.3	286	24	97	10	52	7
2	8	2.2	0.4	54.1	2.8	1.0	0.3	258	27	130	18	32	11
3	8	2.0	0.7	49.0	1.4	0.1	0.3	140	17	130	14	32	6
4	8	1.5	0.2	47.9	1.0	0.1		71	28	136	10	26	3
5	8	1.5	0.7	47.2	0.5	0.1		71	17	135	4	25	4
6	8	0.9	0.3	47.0	0	0.1		63	11	111		25	3
						Dog B—Cortisone							
1	5	2.3	0.7	111.6	23.0	2.4	0.6	309	17	94	16	51	6
2	8	2.5	0.3	60.7	3.1	1.1	0.4	243	25	119	11	33	9
3	8	2.5	0.6	51.8	2.3	0.2	0.2	105	38	126	14	29	6
4	8	2.0	0.5	48.3	1.2	0.1		78	16	128	10	27	7
5	8	1.8	0.3	48.2	1.3	0.1		45	16	128	9	26	7
6	8	1.5	0.4	47.3	0.5	0.1		43	15	126	11	24	8

Dog A—10 control and 7 cortisone studies. Dog B—4 control and 6 cortisone studies. Since there was no relationship between the changes produced by cortisone and the duration of cortisone administration, the cortisone figures represent the mean values for all studies done during the six weeks of cortisone administration.

creozymin-secretin stimulation was greatest in the first two collection periods and averaged 2.0 to 2.3 ml. per min. (Table I, Fig. 4A). During cortisone administration the rate of flow of pancreatic juice in both dogs following pancreozymin-secretin stimulation was increased in all collection periods

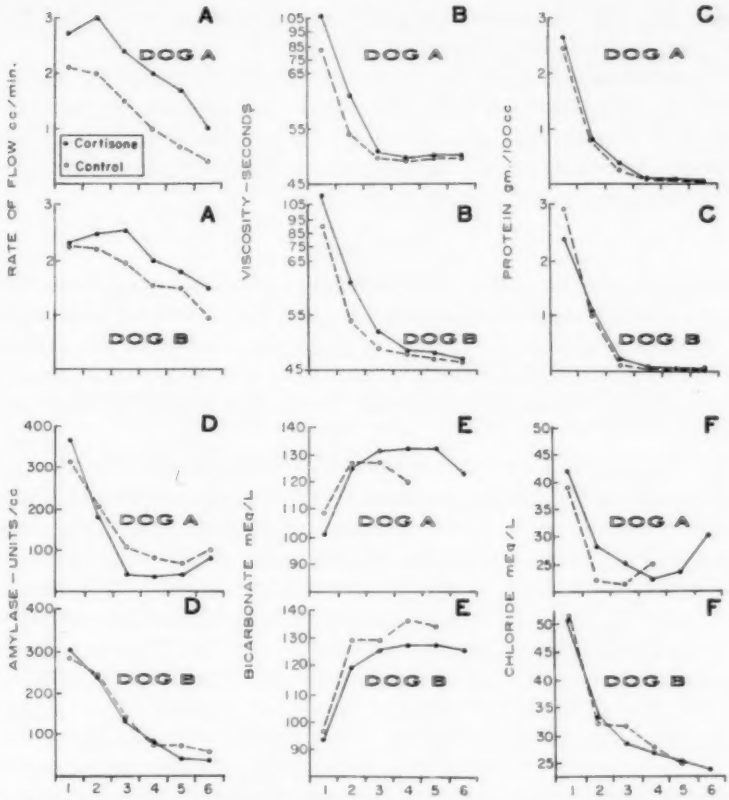

FIG. 4. Effect of cortisone on the pancreatic juice produced by simultaneous pancreozymin and secretin stimulation. Mean results of ten control and seven cortisone studies in dog A and four control and six cortisone studies in dog B. The numbers on the horizontal axis represent the individual collection periods. Amylase units have been multiplied by 10^{-3}.

except collection #1 of dog B.[5] These increases ranged from 14 to 100 per cent greater than corresponding control values.

The viscosity of the initial 5 cc. of pancreatic juice was highest and most variable. During the cortisone period the viscosity increased in the first two collections of pancreatic juice of dog A and the first three collections of dog B[6] (Table I, Fig. 4B). Viscosities in the third and subsequent collections de-

[5] P level of .02 to .001 for all collection periods in dog A and 0.2, 0.2, 0.1, 0.3 and 0.02 for period 2 through 6 in dog B.

[6] P levels of .1, .001 (dog A) and 0.2, .02, .02 (dog B).

creased to a range between forty-seven and fifty-two seconds, generally less than produced by meal stimulation (Fig. 1C).

In contrast to meal stimulation, protein and amylase activity rapidly decreased in the pancreatic juice after pancreozymin-secretin stimulation. Despite the higher flow rates produced by pancreozymin-secretin during cortisone administration the bicarbonate and chloride concentrations were similar to control values (Table I, Fig. 4C–F).

DISCUSSION

The most notable change produced by cortisone was an increase in the rate of excretion of pancreatic juice following both meal and pancreozymin and secretin stimulation. This was of particular interest in the pancreozymin-secretin experiments since the maximum flow response to pancreozymin-secretin stimulation determined during the control period was exceeded.

The viscosity of pancreatic juice following meal stimulation was not significantly altered during cortisone administration. Moreover, the finding that viscosities of the first 5 cc. collection samples were lower than the corresponding control collections suggested that the viscosity of duct contents in the resting gland was not increased by cortisone. The viscosities in the first two or three collections of pancreatic juice following pancreozymin-secretin stimulation were definitely increased during cortisone administration. In the preliminary stimulation studies on these dogs the addition of pancreozymin to secretin produced a 25 per cent increase in the viscosities of the first three collection samples. The further increase in viscosities observed during cortisone administration possibly was due to an enhanced pancreozymin effect on viscosity of the newly formed secretions rather than an increase in viscosity of duct contents in the resting gland. Although viscosity increased during cortisone administration in the pancreozymin-secretin stimulation studies (Fig. 4B), the viscosity of these secretions (excluding collection #1) was no greater than that which normally followed eating a meat meal (Fig. 1C).

During cortisone administration collections of pancreatic juice with similar protein concentrations had definitely higher viscosities (Fig. 3, 4B–C). In an attempt to explain this observation the relationship between viscosity and the molecular weight and concentration of various proteins was examined. The viscosities of isotonic sodium chloride solutions containing increasing concentrations of human albumin (mol. wt. 39,000) gamma globulin (mol. wt. 180,000) and fibrinogen (mol. wt. 450,000) were measured. As shown in Figure 5, the viscosity increased as both the concentrations and molecular weights of the proteins increased. Thus the changes observed in the dogs suggested that a protein of higher molecular weight or

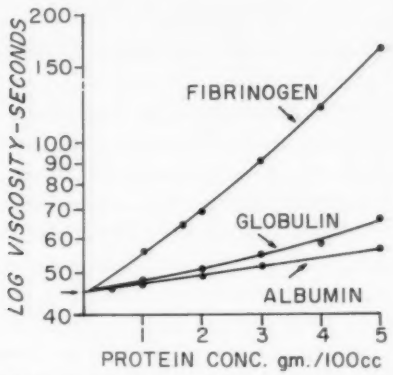

FIG. 5. The viscosity of increasing concentrations of albumin (mol. wt. 39,000) gamma globulin (mol. wt. 180,000) and fibrinogen (mol. wt. 450,000). The arrow on the vertical axis indicates the viscosity of isotonic sodium chloride.

some new or altered non-protein substance had appeared in the pancreatic juice during cortisone administration.

Cortisone also changed the relationship between the rate of flow and the viscosity of individual collections of pancreatic juice (Fig. 2, Table I and Fig. 4A–B). Followed both meal and pancreozymin-secretin stimulation flow rates were higher while viscosities remained unchanged or increased. According to the physical laws of viscous flow for solid tubes developed by Poiseulle the P (driving pressure) in a tube of length L is proportionate to k (KV · F) where KV = viscosity and F = rate of flow. For secretions of similar viscosity (KV), an increased rate of flow (F) would require an increased head of pressure (P) at the driving end of the tube—the individual acinus in the case of the pancreas. Although neither dog developed pancreatic lesions, it is conceivable that in susceptible individuals or species chronic increases in the rate of flow (F) and the driving pressure (P) resulting from continuous administration of steroids could cause dilatation of the pancreatic acini or collecting ducts.

Studies in man by Dreiling *et al.* (15) indicated that acute administration of ACTH or adrenal corticosteroids decreased the response of the pancreas to secretin as reflected by the rate of flow, bicarbonate concentration and amylase activity of duodenal collections. In the present experiments direct cannulation of the main pancreatic duct of dogs during cortisone administration showed a definite increase in the rate of flow of pancreatic secretions. Bicarbonate content was not depressed. On the other hand, amylase activity of the collected secretions following meal stimulation was consistently less. Following pancreozymin-secretin stimulation amylase activity in pancreatic juice was unchanged from the control values.

SUMMARY

The effect of cortisone administration on pancreatic function was studied by direct cannulation of the main pancreatic duct in dogs prepared with chronic duodenal fistulae. Pancreatic flow was stimulated in control and cortisone periods by giving a meat meal and by the simultaneous intravenous injection of pancreozymin and secretin. During cortisone administration the rate of excretion of pancreatic juice was increased following both of these stimuli. The viscosity of pancreatic secretions following meal stimulation was not changed during cortisone administration. Cortisone did increase the viscosity of pancreatic juice following pancreozymin-secretin stimulation. However with the exception of the first 5 cc. of pancreatic juice excreted the viscosities, although greater than the corresponding control values, did not exceed viscosities which normally resulted from meal stimulation. The changes observed in the rate of flow and viscosity of pancreatic juice were discussed in relation to the pathological changes observed in steroid-induced pancreatitis.

ACKNOWLEDGMENT

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EXAGGERATED NATRIURESIS IN PATIENTS WITH CONGENITAL HEART DISEASE[1]

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In congestive heart failure, impaired cardiac function results in reduced excretion of sodium by the kidneys. Under other circumstances, heart disease results in alterations of renal function in the opposite direction; i.e., sodium excretion is enhanced (1 to 4). For example, an exaggerated natriuresis during water diuresis has been observed by Werko and his associates (1, 2) in patients with rheumatic heart disease and hypertension. The mechanism whereby changes in cardiac function produce changes in renal function is unknown, but it has been suggested that there is a receptor organ within the central circulation which responds to alterations in pressure or volume and initiates the renal response (5).

Patients with congenital heart disease were selected as subjects for the current study because congenital abnormalities of the heart are associated with a variety of alterations in pressure and flow within the various segments of the central circulation. If alterations in sodium excretion were mediated by hemodynamic alterations in some specific part of the central circulation, it might be possible to localize this area by the study of appropriate congenital heart lesions, especially before and after surgical correction. In the present study, a diuresis test similar to that of Bucht (1) was applied to the study of sixteen patients with congenital heart disease and seven normal control subjects. The control subjects were investigators and hospital patients free of cardiovascular and renal disease. No patient was in congestive failure. All subjects in both groups were on a normal diet without salt restriction.

METHODS

The technic employed is illustrated by Figure 1, which presents the results of a typical diuresis study on a normal control subject. All experiments were performed in the morning in a fasting state; the subjects had been forbidden to eat or drink after midnight. One

[1] Supported by grants from the Life Insurance Medical Research Foundation and the American Philosophical Society.

[2] This work was done during the tenure of a Research Fellowship of The American Heart Association.

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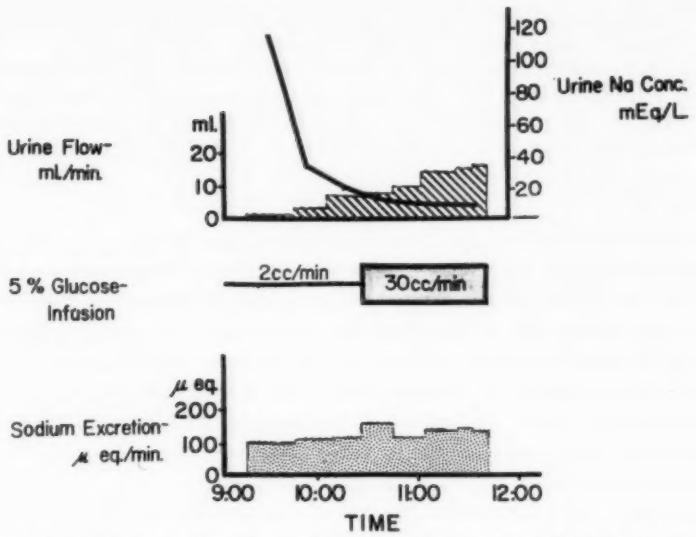

FIG. 1. Water diuresis in a normal subject. During the control infusion of 5 per cent glucose at 2 ml. per min., there was a gradually rising urine flow (cross-hatched blocks in upper figure) accompanied by a falling urine sodium concentration (bold line in upper figure). Following the onset of rapid infusion, the urine flow continued to increase to about 15 ml. per min., and the urine sodium concentration decreased further. The sodium excretion in μEq. per min. remained nearly constant as a result of the reciprocal changes in urine flow and sodium concentration.

The increase in average urine flow (Δ Flow) was 10.3 ml. per min., and the Δ Na excretion was 25 μEq. per min.

hour before the beginning of the experiment, the subject drank 250 ml. of water to insure a measurable initial urine flow. In the case of the female patients, a soft rubber catheter was inserted at this time and taped to the thigh. An intravenous infusion of 5 per cent dextrose in water was begun and regulated by drip to 2 ml. per min. during a 90 to 120 minute control period. Thirty minutes after the start of the infusion, the bladder was emptied by voiding in the male and by catheter and manual pressure in the female subjects. Three 30-minute control urine collections were then obtained. Following this, the infusion rate was increased to between 25 and 35 cc. per min. by the use of a syringe infusion pump.[4] This period of rapid infusion continued for eighty minutes, during which time one or more urine samples were collected every twenty minutes.

The volume of each urine sample was measured and the sodium concentration determined with a Baird flame photometer.[5] Urine flow and sodium excretion rates were calculated for the three control periods and for all but the first of the rapid infusion periods. The first 20 minutes of the rapid infusion period represented a period of equilibration and readjustment, and for this reason the urine sample collected during this

[4] Baltimore Biological Laboratory, Model 07-40-SS-50.

[5] Baird Associates, Inc. #1186-1.

period was omitted from the final calculations. Blood was also drawn for determination of serum sodium concentration at the beginning and end of the experiment.

The average urine flow during the three control periods was subtracted from the average urine flow during the three rapid infusion periods, and the result expressed as Δ Flow (ml. per min.). A similar calculation was performed for the the sodium excretion, and the result expressed as Δ Na (μEq. per min.).

RESULTS

A representative abnormal response is depicted in Figure 2. The subject of this experiment was a twenty-five-year-old white female who was found to have the typical murmur of a patent ductus arteriosus on routine physical examination. She was completely asymptomatic, with essentially unlimited exercise tolerance. Chest roentgenogram, electrocardiogram, and routine blood and urine examinations were normal. During the period of rapid infusion, there was a marked increase in sodium excretion, with a Δ Na of 239 (μEq. per min.). The Δ Na (μEq. per min.) for the normal experiment depicted in Figure 1 was 25.

The values of Δ flow and Δ Na for all the patients are given in Table I. Figure 3 is a cumulative frequency graph of values of Δ Na and clearly demonstrates a difference between the normal subjects and the patients with congenital heart disease. Ninety per cent of the normal control subjects had a Δ Na of less than 60 (μEq. per min.) while 75 per cent of the patients with heart disease exceeded this Δ Na value. The difference be-

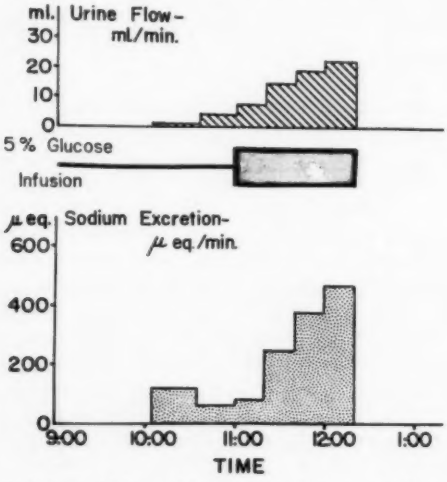

FIG. 2. Water diuresis in a subject with a patent ductus arteriosus. Rapid glucose infusion produced an increase in urine flow (Δ Flow = 17 ml. per min.) and a striking increase in sodium excretion (Δ Na = 239 μEq. per min.).

TABLE 1

The Effect of Infusion on Urine Flow and Sodium Excretion

	Patients	Experiments	Δ Flow (ml./min.)		Δ NA (μEq./min.)	
			Average	Range	Average	Range
Normal controls	7	10	11.0	(.1 to 24.5)	46	(13 to 190)
Congenital heart disease	16	24	16.7	(4.3 to 26.0)	170	(−19 to 627)
Tetralogy of fallot	2	3	9.7	(7.2 to 13.4)	81	(−19 to 152)
Pulmonary stenosis	2	2	15.9	(13.2 to 18.5)	314	(209 to 627)
Pulmonary hypertension	1	1	4.3		65	
Coarctation of aorta	3	6	12.3	(5.4 to 20.0)	90	(5 to 214)
Atrial septal defect	3	3	12.3	(9.3 to 16.5)	240	(53 to 441)
Patent ductus arteriosus	5	9	17.5	(11.6 to 26.0)	211	(78 to 512)

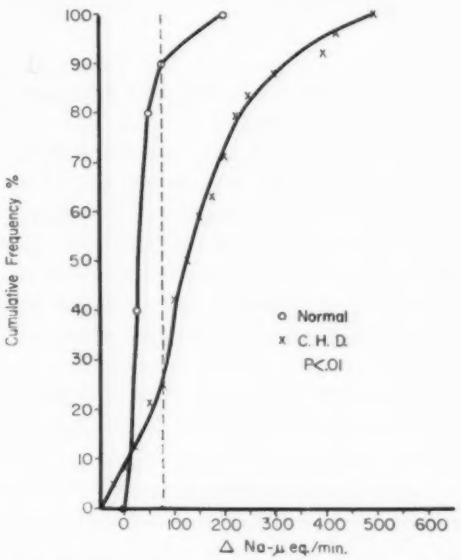

FIG. 3. The cumulative frequency of the Δ Na values in the two groups of patients is shown in the two curves. It can be seen that 90 per cent of the normal individuals (open circles) had Δ Na values less than 60 μEq. per min., while 75 per cent of the patients with congenital heart disease had Δ Na values higher than this figure. The difference between the the two groups is highly significant.

tween the two populations was highly significant ($p < .01$), with respect to Δ Na. There was no significant difference with respect to Δ flow.

In Figure 4 Δ flow is plotted against Δ Na with each point representing a single experiment. High and low values for Δ flow are seen in both groups, but the separation of the two populations with respect to Δ Na is clearly

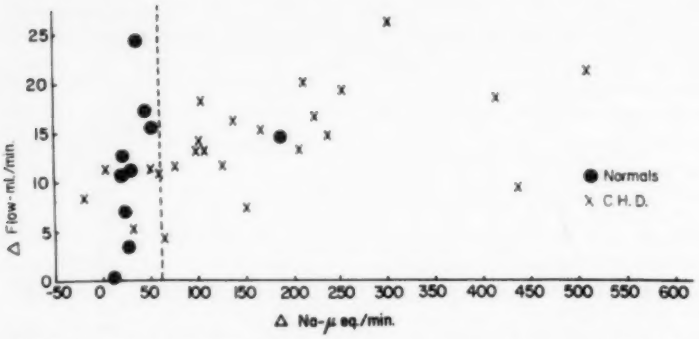

FIG. 4. The Δ Flow and Δ Na values for each experiment are represented by single points. All of the normal control subjects (solid dots) except one have Δ Na values less than 60 μEq. per min.

seen. No significant correlation could be found between Δ Na and Δ flow in either the normal or patient groups.

Serum sodium fell during the period of rapid infusion in all the subjects. The mean values of serum sodium for the normal group before and after infusion were 143 and 135 mEq./L. respectively. Comparable values for the congenital heart disease group were 142 and 130 mEq./L. The post infusion serum sodium was significantly lower in the patient group ($p < .01$). The infusion produced a drop in hematocrit of from one to five points in the three normal subjects and four patients upon whom this measurement is available.

Seven patients with congenital heart disease were restudied after surgical correction of the cardiac lesions (Table II). In four patients, the surgery was

TABLE II

The Effect of Surgery on Natriuresis

Diagnosis	Operation	Pre-operative Δ Na (μEq. min.)	Post-operative Δ Na (μEq./min.)	Time After Operation
Coarctation of aorta + aortic insufficiency	Resection of coarctation	34	51	14 days
Coarctation of aorta	Resection of coarctation	5	214	15 days
Patent ductus arteriosus	Ductus divided	239	512	56 days
Patent ductus arteriosus	Ductus divided	105	302	12 days
Patent ductus arteriosus	Ductus divided	139	168	20 days
Patent ductus arteriosus + ventricular septal defect	Ductus divided	100	78	5 months
Tetralogy of fallot	Blalock-Taussig anastomosis	−19	109	16 days

considered to be corrective, in that the cardiovascular anatomy and physiology were normal after the operative procedure. Patent ductus arteriosus probably represents the simplest and the most easily corrected of the abnormalities studied. In all three patients with patent ductus arteriosus as the only abnormality, the Δ Na was greater in the postoperative study than in that done before operation.

DISCUSSION

The present investigation has clearly demonstrated that patients with congenital heart disease have an exaggerated natriuresis similar to that demonstrated by others in patients with rheumatic heart disease and hypertension (1, 2). This abnormal response was seen in all types of congenital heart disease studied, and there did not appear to be any relation between the magnitude of the natriuresis and the functional abnormality of the circulation.

An increase in sodium excretion with infusion might be attributed to the osmotic load provided by the glucose, but this mechanism can not be invoked to explain the difference between the normal subjects and the patients with congenital heart disease, since the osmotic load infused was approximately equal in both groups. The natriuretic response to the infusion of salt free fluid in patients with congenital heart disease might be the result of either changes in renal plasma flow, glomerular filtration rate or tubular reabsorption. Altered tubular function could be either a direct consequence of altered renal circulation or related to the inappropriate response of a volume or osmoreceptor operating via hormonal or neural pathways. The simpler and more direct mechanisms involving changes in renal hemodynamics must be considered first. An increase in renal plasma flow, glomerular filtration rate, or filtration fraction during the period of rapid infusion could account for the observed increase in sodium excretion. It is possible that a borderline depression of renal perfusion exists in the patients with congenital heart disease (6) and that a slight increase in glomerular filtration rate and hence sodium excretion occurs with rapid infusion.

The fall in serum sodium in all subjects can be attributed to the expansion of the plasma volume by the infusion of sodium free fluid. The greater fall in the patients with congenital heart disease can be explained by the greater urinary loss of sodium which occurred in these individuals.

The persistence of the natriuretic response after corrective surgery is difficult to explain. It is possible that congenital heart disease is associated with an independent congenital abnormality of renal function. It seems more likely that the renal circulation does not return to normal immediately following the restoration of a normal central circulation. Some support for this theory is found in the work of Werko who measured renal function before and after surgical correction of congenital heart lesions (6).

SUMMARY

1. The excretion of sodium and water in the urine was measured before and during a rapid intravenous infusion of 5 per cent dextrose in seven normal control subjects and sixteen patients with congenital heart disease.

2. Both groups exhibited a water diuresis, but the patients with heart lesions excreted significantly more sodium.

3. There was no correlation between the type of congenital lesion and the magnitude of the natriuresis.

4. The altered sodium excretion persisted after corrective surgery in four patients.

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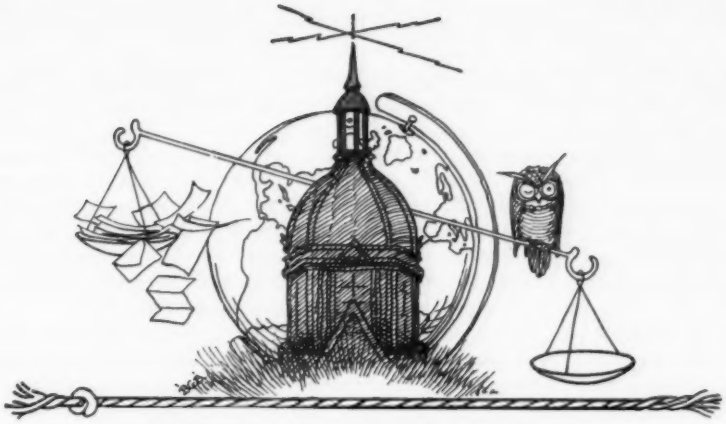

BOOK REVIEWS

The Cervix Uteri and its Diseases. By FREDERIC FLUHMANN. 556 pp., $14.00. W. B. Saunders Co., Philadelphia, Pa., 1961.

Among the most important publications pertaining to the uterine cervix within the last few decades have been those of Dr. Fred Fluhmann. Thus it can be no surprise to those of us who know him as an expert student of his specialty to find that his recent text is a really outstanding contribution of gynecology, histology and pathology. The author's personality has been transmitted to his text, which indicates an immensely pleasant and appealing approach to the subject matter.

In slightly more than 500 pages, with slightly fewer but uniformly excellent illustrations, a true panorama of the cervix is presented. In his preface the author notes the extreme importance of the cervix uteri in the female patient; indeed cervical cancer is the most common cause of fatal genital malignancy. It is pointed out that certain views and introduced terms have been advanced as a result of personal investigation, although not necessarily in conformity with past accepted theories. While it is likely that certain of the "old guard" members of this specialty will look down at his suggestions, certainly his stimulating ideas based on sound clinical and academic studies are appealing to many of us.

In brief, this text is divided into six basic sections. The normal cervix (1) includes anatomy, histology, and physiology. Diagnostic procedures (2), and congenital anomalies and traumatic and functional disorders (3) are presented in complete conventional fashion, as is the section on inflammatory diseases (4). The section of new growth (5) (although the title is not particularly appealing), is one of the best treatises on cervical malignancy we have encountered. Full coverage of radiological and surgical treatment is included, along with the excellent operative technique depicted by Parsons and Uhlfelder. The author echoes the sentiment of Meigs that radiation and operative treatment should be complementary rather than antagonistic. Section 6 deals with pregnancy and the many changes that may occur in the cervix including true neoplasia and various pseudoneoplastic changes. An excellent review of the incompetent cervix is included along with operative approach in the gravid and non-pregnant state (surgery is preferable in the latter if possible).

We should reiterate that there are a few opinions that may not conform to a

majority. Nevertheless, the expressed and previously published ideas that endocervical glands are in reality clefts is different, and the author offers strong and rather convincing support with excellent diagrams and figures.

Whether the term squamous prosoplasia will replace the time honored metaplasia seems doubtful, as does routine usage of the term cervical mucus polyp. The concept that normal cervical cells may differentiate into either squamous or columnar pattern is unusal, and this concept is utilized to explain the genesis of the admittedly uncommon "adenoepithelioma" of the cervix. However, it would take a braver soul than I to disagree with anyone who has so dedicated himself to cervical histology and disease.

We would like to venture a prediction that this text will become a classic in its field, although obviously the field is limited. However, there is no more important area in gynecological practice, and one can be sincerely advised that there is no better means of education than study of this treatise. To the author go our sincerest congratulations for a real masterpiece.

EDMUND R. NOVAK

Microbial Reaction to Environment; Eleventh Symposium of The Society for General Microbiology. Edited by G. G. MEYNELL and H. GOODER. 416 pp., $7.50. Cambridge University Press, New York, N. Y., 1961.

This published symposium is a detailed presentation of the behavior of microorganisms under varying artificial conditions. The principle effort to relate changes in microorganisms, attributed to environment, to the process of infection, was made by G. G. Meynell. This paper, titled "Phenotypic Variations and Bacterial Infection," should have been given a more prominent place in the published symposium, and should have been the lead for a greater effort to relate biochemical and morphological microbial variations to other phenomena. There obviously is extraordinarily exciting and new information brought together in this book. Illustrative of the difficulties involved in translating information, however, is the lack of a paper, except Meynell's integrating basic studies on bacteriology with other areas of cellular biology and infectious disease. Symposia should serve not only to reacquaint those already knowledgeable with what they know, but should also serve to indicate the relationships of one body of knowledge with another. There are few with the ability to so broaden their perspective and the perspective of others.

As presented, this symposium will be of little value except to individuals specifically investigating the field of bacterial morphology and biochemistry. It is to be hoped that a few others may find ways to integrate such information with broader biological concepts.

LEIGHTON E. CLUFF

A Synopsis of Contemporary Psychiatry, 2nd edition. By GEORGE A. ULETT AND D. WELLS GOODRICH. 309 pp., $6.50. The C. V. Mosby Co., St. Louis, Mo., 1961.

This book has the format of a compact car. It fits in the doctor's uniform pocket. Content is abbreviated for economy in words and size. Reviewing the contents, one finds the usual introduction, history and diagnostic procedures. There is a chapter on the psychodynamic concept of personality development, followed by clinical syndromes which are presented very much in the thinking of the American Psychiatric Association book of nomenclature. Special chapters on mental deficiency and psychiatry conclude the descriptive material.

Therapeutic measures cover almost 100 total pages. This application of space to

therapy is admirable. The only criticisms of the therapeutic part, ones which this writer usually finds applicable to psychiatric textbooks, are that there is not enough specific material on psychotherapy. The descriptions and techniques in psychotherapy are too generalized. A few brief, concise examples would be more helpful than several pages of description.

Chapter twenty-five, which concerns the management of suicidal patients, is well presented and certainly of great importance. The only criticism is that this two page chapter should cover a great deal more material. On the other hand, the chapter on chemotherapy, which covers approximately forty pages, is in the opinion of this reviewer, out of proportion to the total importance of such a specific method of therapy. It is true that the author gives a table of various useful drugs (some of them not so useful) available at the time of publication. Since their table was published, undoubtedly at least half as many again are available, but the basic chemical species of drugs are still very much in use clinically. Specifically, the authors would do more service by stressing the dangers of chemical therapy, which is a very widespread practice among both psychiatrists and other physicians in practice. For example, the danger of addiction to meprobamates, the real danger of the so-called safe, doriden type compounds, the danger of depressive reactions from trilafon and similar compounds, as well as the reserpine type compounds, is not sufficiently stressed. The amphetamines are not mentioned as sources of serious habituation.

In the space allotted the discussion of forensic psychiatry is excellent and the description, and explanation of character disorders very well presented.

The only other criticism involves the psychophysiological reactions in which personality types are cited for certain or specific types of illness. This does not make sense to this reviewer.

In summing up, this book is a very useful book for the intern, the psychiatric resident and others in allied fields interested in the clinical aspects of psychiatric treatment and practice. It is most difficult to write a concise, informative book on the subject of psychiatry for the practitioner. This book has succeeded. The reviewer is critical of certain aspects, but in general commends this book for its purpose.

WILLIAM T. DIXON

Symposium on Mammalian Genetics and Reproduction. Reprinted from Journal of Cellular and Comparative Physiology, Vol. 56, Supplement 1, November, 1960. Published by The Wistar Institute of Anatomy and Biology, Philadelphia, Pa., 1960.

This volume is a report of the proceedings of the thirteenth symposium sponsored by the Biology Division of the Oak Ridge National Laboratory. Included are twelve papers presented at Gatlinburg, Tennessee, April 4–7, 1960. Since they may reflect alternate interpretations, the inclusion of the informal discussions, which followed each formal paper, provides a valuable adjunct. Consideration of each paper is out of the question here; a tally of the contents must suffice to indicate the breadth of the symposium:

Austin: Anomalies of fertilization leading to triploidy; Braden: Genetic influences on the morphology and function of the gametes; Mintz: Embryological phases of mammalian gametogenesis; Gruenberg: Developmental genetics in the mouse, 1960; Hollander: Genetics in relation to reproductive physiology in mammals; Owen: Current status of mammalian immunogenetics; Gluecksohn-Waelsch: The inheritance of hemoglobin types and other biochemical traits in mammals; Chase and Mann: Phenogenetic aspects of some hair and pigment mutants; Griffen: Mammalian pachytene

chromosome mapping and somatic chromosome identification; Falconer: The genetics of litter size in mice; Wright: The genetics of vital characters of the guinea pig; Russell and Russell: Genetic analysis of induced deletions and of spontaneous nondisjunction involving chromosome 2 of the mouse.

It is worth noting that the major emphasis in this symposium is on the mouse, though the several papers often have a comparative view. Only the paper by Sewall Wright, an extensive review of the genetics of morphological abnormalities and quantitative variants in growth, fecundity and mortality in the guinea pig, deals exclusively with another mammal. The impression is gained that the mouse continues to perform the rôle of a surrogate human being in mammalian genetics, especially in developmental genetics.

As Alexander Hollaender indicated in his introduction, the purpose of the symposium was to indicate the present status of the field and the lines of prospective research. Although one might have wished for a discussion of such matters as sex ratio, isozymes, etc., the objectives were adequately accomplished, both in the formal presentations and in the free discussions. As a view of non-human, mammalian genetics, this symposium is a valuable contribution.

WILLIAM J. YOUNG

Attenuated Infection. The Germ Theory in Contempory Perspective. By HAROLD J. SIMON. Forewords by Rene J. Dubos and Walsh McDermott. 349 pp., $10.00. J. B. Lippincott Co., Philadelphia, Pa., 1960.

The formulation of the germ theory of disease in the latter half of the nineteenth century found expression in the writings of Huxley who portrayed nature as a fierce battlefield whose participants were "red in tooth and claw". Such an approach in which man, the host, was depicted in a state of ceaseless struggle with microbial parasites served a certain usefulness and motivated many of our earlier advances in the control of the most severe infectious diseases. The jungle analogy however has its limitations, and a more recent approach to our understanding of infectious disease finds a scholarly, detailed and well-written presentation by Dr. Harold J. Simon in *Attenuated Infection*. In this work, largely conceived and written while the author was in residence at the Rockefeller Institute, the thesis is carefully developed and the evidence impressively marshalled to support the concept that peaceful coexistence between host and parasite is the usual state of affairs, and disease the exception. Thus *infection* (which is defined as the presence of microorganisms in host tissues) is a "necessary but not a necessarily *sufficient* condition" for the occurrence of disease (that is, actual damage to host tissues). The concept of attenuated infection embraces the carrier state, microbial persistence and true latency (which differ in the extent to which the microorganism may readily be cultured from the host and in the state of virulence and of metabolic activity of the microorganism).

Attenuated infection is of great significance not only because it is the most common state of host-parasite affairs but particularly because of the metamorphosis to progressive infection and disease which may occur when the balance of the host-parasite relationship is altered by such agents as antibiotics, corticosteroids, radiation, nutritional aberrations, and the like. Indeed the phenomenon of microbial persistence has emerged in large part as a result of the use of antibiotics and following their discontinuation in a given case may be followed by the re-emergence of active disease. The practical importance of this concept is reflected in the frequency with which the so-called "normal

flora" are emerging as significant causes of disease in the susceptible hospital patient population.

The concepts described are not "original" to the author, and indeed the influence of Drs. Dubos and McDermott for example, are evident. (Appropriately these two distinguished investigators have each contributed a succinct and pertinent foreword.) The author however has systematized these concepts, carefully defining his terms and has assembled a wide diversity of evidence from many fields. The argument is well presented in the introduction and again in the epitome which constitutes the last section of the book. In between are sections dealing with the general aspects of the subject as manifest in almost every biologic system, the mechanisms and dynamics of attenuated infection in tissue culture systems, and finally a consideration of attenuated infection in man including the influence of cultural factors and of man's higher integrative functions. Particular emphasis is placed on the medical, epidemiologic and ecologic aspects.

As a final aside the author reminds us that writers such as Huxley attempted to justify the socio-political policies of the nineteenth century—the "ruthless subjugation of the technologically under-developed peoples by the colonial powers"—on the basis that fierce competition, the law of the jungle and struggle for existence are fundamental rules of nature. Dr. Simon argues that "biologic interactions in nature commonly take the form of mutual aid (cooperation) or mutual tolerance (peaceful coexistence)". War is therefore *not* biologically determined and the causes of war must be sought elsewhere. The author admits his own susceptibility to cultural influences (as with Spencer and Huxley), but by basing his arguments on well-established biologic principles at least makes a strong case that ceaseless struggle between men cannot be justified as inevitable on biologic grounds.

Whether or not one accepts this conclusion the work remains a well-written interpretation of the "germ theory in contemporary perspective" and a useful assemblage of pertinent facts from a variety of sources.

JOSEPH E. JOHNSON, III

BOOKS RECEIVED FOR REVIEW

Appraisal of Current Concepts in Anesthesiology. Edited and assembled by JOHN ADRIANI. 279 pp., $7.75. The C. V. Mosby Co., St. Louis, Mo., 1961.

Clinical Orthopaedics, No. 20. Disorders of the Shoulder Joint. Edited by ANTHONY F. DEPALMA. 272 pp., $7.50 single copy. J. B. Lippincott Co., Philadelphia, Pa., 1961.

Fourth National Cancer Conference Proceedings, 1960, University of Minnesota, Sept. 1960. Sponsored by American Cancer Society, Inc. and National Cancer Institute, U. S. Public Health Service. 774 pp., $9.00. J. B. Lippincott Co., Philadelphia, Pa., 1961.

Mechanisms of Disease. An Introduction to Pathology. By RUY PEREZ-TAMAYO. 512 pp., $14.00. W. B. Saunders Co., Philadelphia, Pa., 1961.

Minds That Came Back. By WALTER C. ALVAREZ. With an introduction by Hervey M. Cleckley. 384 pp., $5.95. J. B. Lippincott Co., Philadelphia, Pa., 1961.

Nerves, Brain and Man. By JOHN GRAYSON. 243 pp., $5.00. Taplinger Publishing Co., New York, N. Y., 1961.

Origin and Nature of Cancer. A Comprehensive and Unified Biologic Theory of Cancer. By ALEXANDER H. KENIGSBERG. 144 pp., $4.00. Twayne Publishers-Bookman Associates, New York, N. Y., 1961.

Pathology, 4th edition. Edited by W. A. D. ANDERSON. 1389 pp., $18.00. The C. V. Mosby Co., St. Louis, Mo., 1961.

Radiographic Anatomy of the Human Skeleton. By W. H. JOHNSON and J. A. KENNEDY. 280 pp., $10.00. E. & S. Livingstone Ltd., Edinburgh and London, 1961. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

The Stages of Human Development Before Birth. An Introduction to Human Embryology. 684 pp., $23.00. W. B. Saunders Co., Philadelphia, Pa., 1961.

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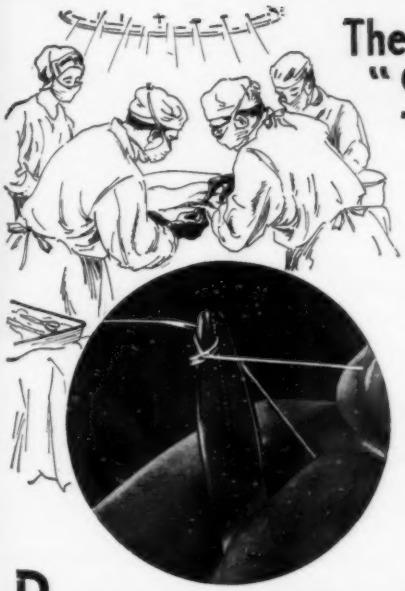

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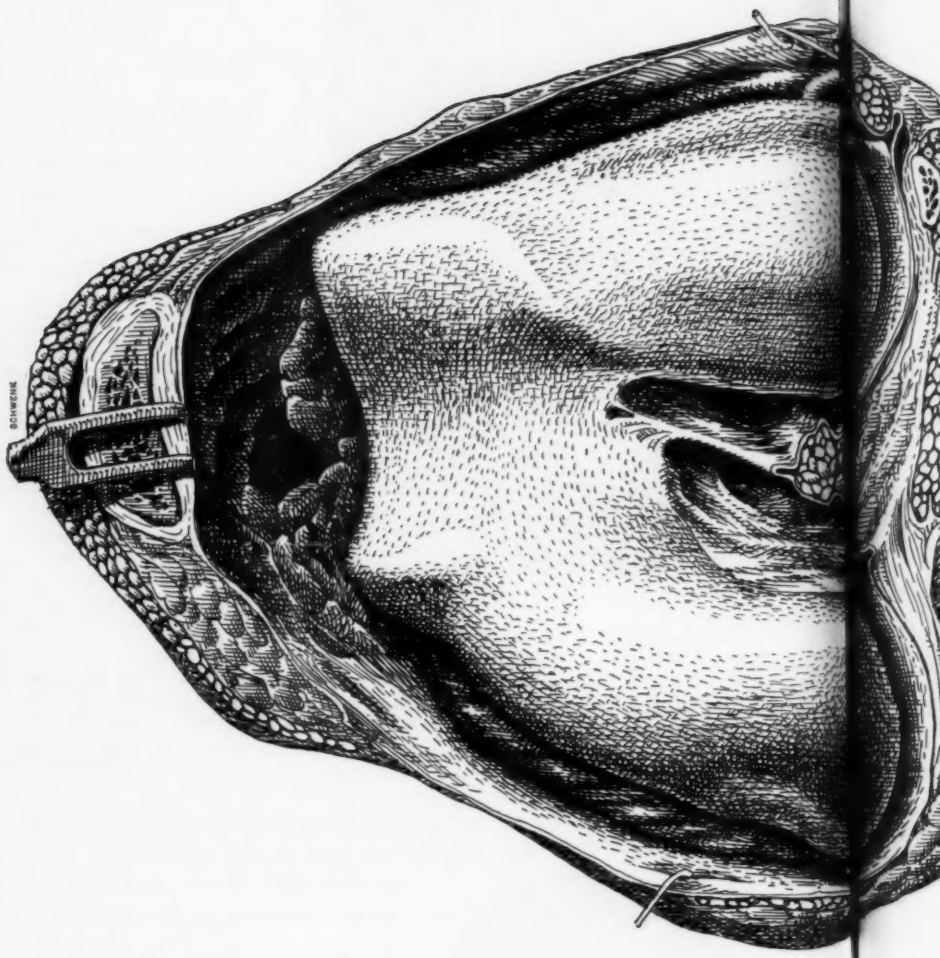

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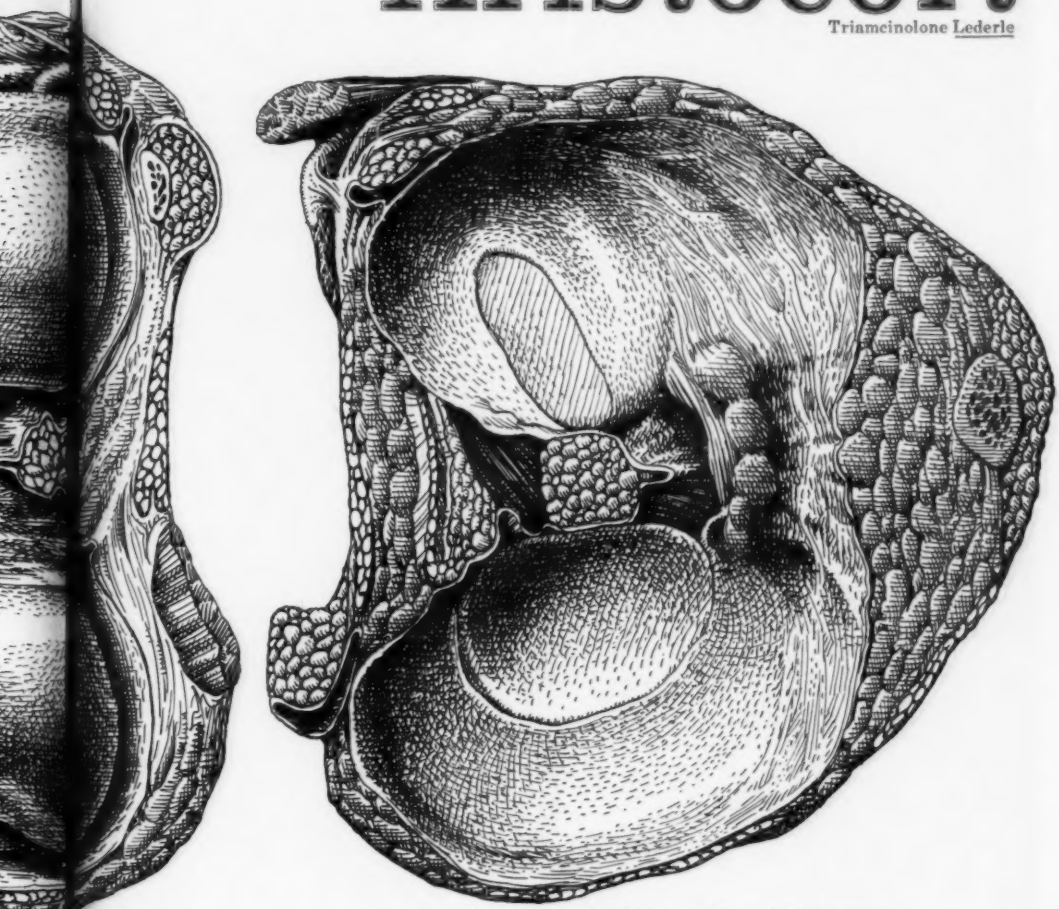

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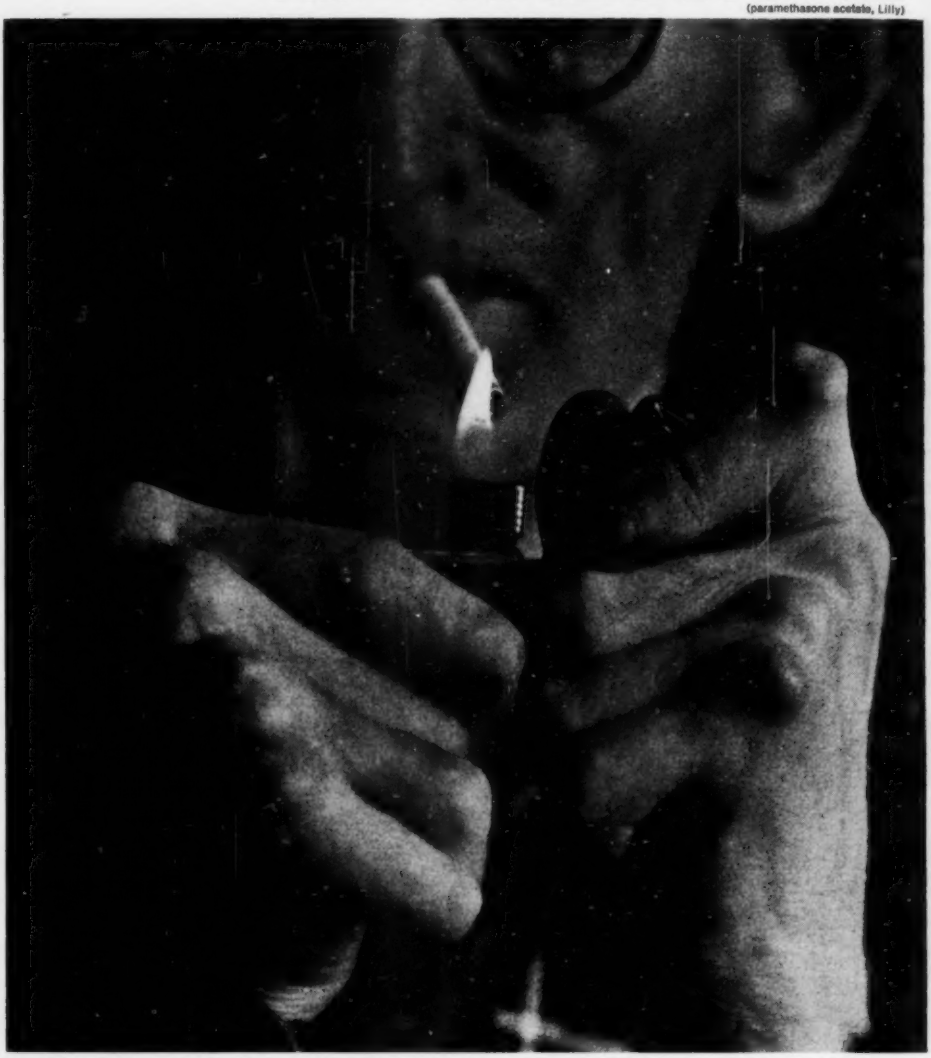

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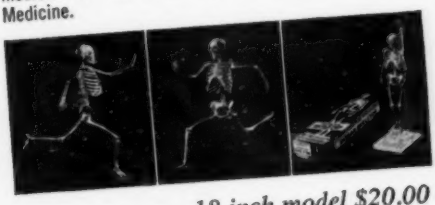

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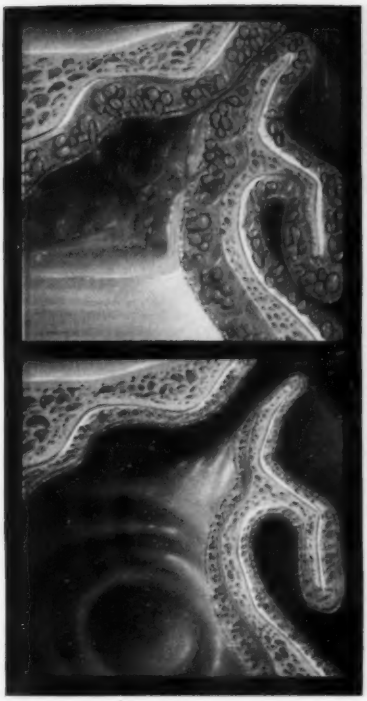

The clogged sinus
In sinusitis, the mucous membrane becomes hyperemic and edematous, lymph glands and goblet cells hyperactive. Ostium is closed by edema and secretions cannot drain freely.

The normal sinus
Magnified anatomy of a portion of maxillary sinus showing mucous membrane with cilia and lymph glands. Ostium is normal and patent.

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When there is nasal turgescence, tiny orifices of sinus ostia tend to clog. Neo-Synephrine nasal solutions and sprays reduce edematous tissues on contact to provide prompt relief. As turbinates shrink, obstructed sinus ostia open, drainage and breathing become freer and the boggy feeling of a cold disappears.

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1. Grant, L. E.: Coryza and nasal sinus infections, **Clin. Med. & Surg.** 42:121, March, 1935. 2. Putney, F. J.: Sinus infection, in Conn, H. F. (Ed.): Current Therapy 1952, Philadelphia, W. B. Saunders Company, 1952, p. 110. 3. Simonton, K. M.: Current treatment of sinusitis, **Journal-Lancet** 79:535, Dec., 1959.

earlier detection of peripheral vascular disease

key to improved therapeutic response

In practically all peripheral vascular disease cases where marked occlusion with severe ulceration or frank gangrene has not developed, patients can be assured that excellent treatment is available and many symptoms can be relieved.[1] Routine palpation of peripheral pulses[2] and performance of clinical tests for peripheral arterial disease[3] will help earlier diagnosis. Consequently treatment can be instituted sooner, improving likelihood of a favorable response to therapy.

VASODĪLAN®

Isoxsuprine hydrochloride, Mead Johnson

myo- -vascular relaxant

increases deep peripheral circulation by direct action
...without troublesome side effects

VASODĪLAN'S record of safety and effectiveness in the management of peripheral vascular disease has been established clinically.[4-6] Clarkson and Le Pere report: "With strictly a clinical office approach, isoxsuprine [VASODĪLAN] was used in the treatment of 100 patients with peripheral vascular disorders. Definite clinical improvement was obtained in 89 per cent of these patients."[5] They further state: "In particular, the symptoms of pain, cramping, numbness, and cold were consistently relieved."[5]

Contraindications—There are no known contraindications to oral administration of VASODĪLAN in recommended doses.

Cautions—VASODĪLAN should not be given immediately postpartum or in the presence of arterial bleeding. Parenteral administration is not recommended in the presence of hypotension or tachycardia. Intravenous administration is not recommended because of the increased likelihood of side effects.

Side effects—Few side effects occur when given in recommended doses. Occasional palpitation and dizziness can usually be controlled by dosage adjustment. Single intramuscular doses of 10 mg. or more may result in hypotension or tachycardia.

Dosage and administration—Oral—10 to 20 mg. (1 to 2 tablets) t.i.d. or q.i.d.; I.M.—5 to 10 mg. b.i.d. or t.i.d.

Supplied—10 mg. tablets, bottles of 100; 2 cc. ampuls (5 mg./cc.) for intramuscular use, boxes of 6.

For complete details on indications, dosage, administration and clinical background of VASODĪLAN, see the brochure of this product available on request from Mead Johnson Laboratories, Evansville 21, Indiana.

References: (1) Lieberman, J. S.: GP *21*:133-143 (March) 1960. (2) DeWeese, J. A.: New England J. Med. *262*:1214-1217 (June 16) 1960. (3) Winsor, T.: Peripheral Vascular Diseases: An Objective Approach, Springfield, Illinois, Charles C Thomas, 1959, pp. 457-458. (4) Kaindl, F.; Samuels, S. S.; Selman, D., and Shaftel, H.: Angiology *10*:185-192 (Aug.) 1959. (5) Clarkson, I. S., and Le Pere, D. M.: Angiology *11*:190-192 (June) 1960. (6) Samuels, S. S., and Shaftel, H. E.: J.A.M.A. *17*:142-145 (Sept. 12) 1959.

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